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THE VISUAL SYMPTOMS OF SOME MINERAL DEFICIENCIES ON CHRYSANTHEMUMS

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INTRODUCTION

The study of the effects of nutritional deficiencies in food crops has assumed considerable proportions during recent years and forms the material for a number of monographs (*e.g.* 1, 4, 22, 24). Decorative crops, however, have received comparatively little attention. Work has been planned in these laboratories to investigate this aspect and in particular to examine the effects of mineral deficiencies on those decorative plants which are grown under glass in this country. Preliminary experiments brought out one very important fact. This was that the omission of one element from a nutrient solution frequently induced different effects in different varieties of the same species. The present paper describes the effects associated with specific deficiencies in some of the varieties of chrysanthemums grown commercially under glass.

Laurie and Wagner[15]) describe the general effects of deficiencies of the major elements in the variety Valencia and give more detailed descriptions of the effects due to deficiencies of sulphur, calcium, magnesium, iron and manganese. It appears that they obtained no specific response to the omission of boron. Description and illustrations of the effects are reproduced in Laurie and Kiplinger's book[14]). Ferguson and Wright[6]) working on the effects of boron deficiency in a number of species describe and illustrate the effects on the flowers of one variety — Marie Adelaide.

Hill, Davis and Johnson[12]) found that low potassium reduced the quality of the blooms and they describe the general effects of both potash and phosphorus deficiencies respectively.

Goodwin–Wilson[8]) found that the nutrient requirement of chrysanthemums depended on the variety; in particular slow growing varieties with small hard foliage needed a higher level of nitrogen.

Brooks[2]) reports inconclusive results of the effects of nitrogen, potash and phosphorus.

Among other workers who have investigated this subject Chailakhyan[3]), Gericke[7]), Krawczyński[13]) and Stecher[21]) were mainly concerned with the major elements. Owen[18])[19]) studied the effects of manurial treatment on the mineral constituents of chrysanthemums and Mazaeva[17]) classified chrysanthemums with some other species as being sensative to magnesium deficiency.

EXPERIMENTAL

In the present work plants were grown from cuttings rooted in soil or in pure sand. The roots of those raised in soil were carefully washed before use and it may be stated that these plants did not differ in subsequent reactions from those which had been raised in sand. Once rooted the plants were transferred to about 7 kg of sand contained in 1-gallon glazed earthenware jars each with a drainage hole at the bottom. The holes were fitted with corks each carrying a bent glass tube. The jars could be filled with nutrient solution and emptied at will via the tube. Sand was prevented from running into the tube by a plug of glass wool immediately behind the cork. For nutrients the purest chemicals available were used and the sand was practically pure silica. In the earlier experiments distilled water was used exclusively for preparation of the nutrient solutions. Later demineralised water prepared by the "Deminrolit" apparatus was used but this contained sufficient boron to make the appearance of symptoms uncertain so distilled water was used for treatments in which this particular element was omitted. Hewitt[11]) reported a similar experience.

The composition of the solutions used in 1950 is shown in Table I and the varieties used in Table II. In 1948, only a quarter of the calcium shown in the table was used, nitrogen was at 75 p.p.m. and molybdenum was not included. In 1949 no molybdenum was

included. In the early stages of growth and in the autumn solution was applied two or three times per week. In the summer when growth was rapid it was applied once or twice daily and at this period both nitrogen and potassium concentrations were increased. As soon as a pot was filled with solution above the level of the sand it was drained immediately.

TABLE I

Composition of the nutrient solutions in p.p.m.												
Treatment \ Nutrient	N	P	K	Ca	Mg	S	Na	Cl	B	Mn	Fe	Trace elements
Complete . . .	110	22	166	57	24	32	trace	trace	0.1	0.25	1.5	Li–0.0025
No nitrogen . .	—	22	129	57	24	72	trace	102	0.1	0.25	1.5	Cu–0.015
												Zn–0.015
No phosphorus.	110	—	166	57	24	43	trace	trace	0.1	0.25	1.5	Al–0.01
												Sn–0.015
No potassium ..	110	22	—	57	24	32	98	trace	0.1	0.25	1.5	Ni–0.01
No calcium . .	110	22	166	—	24	32	65	trace	0.1	0.25	1.5	Co–0.01
												Ti–0.03
No magnesium .	110	22	166	57	—	32	46	trace	0.1	0.25	1.5	I–0.02
												Br–0.02
No manganese .	110	22	166	57	24	32	trace	trace	0.1	—	1.5	SiO_2–0.2
No boron . . .	110	22	166	57	24	32	trace	trace	—	0.25	1.5	Mo–0.01

The pH of freshly made solution varied from 5.2 to 5.6 but during use the reaction value tended to rise and periodically 0.1 *N* sulphuric acid was added to keep the value below 7.

In the main the plants were kept in a glasshouse and this had the effect of causing earlier flowering than is the case when the plants are out in the open during the summer months. Routine treatment for pests and mildew was carried out when necessary. All varieties were "stopped" twice and where buds were considered to have developed too early they were taken out and the next ones secured.

Except where the effects of a particular deficiency were required in young plants the respective treatments were started after the plants had become well established in complete nutrient solution.

TABLE II

No	Variety	Colour of blooms	Character of blooms	Elements omitted in		
				1948	1949	1950
1	Rose Harrison	pink	reflexed	N, P, K, Ca Mg, Mn, B	N, P, K, Ca, Mg, Mn, B	Mn, B.
2	Bronze Harrison	light bronze	reflexed	N, P, K, Ca, Mg, Mn, B	—	—
3	Imperial Pink	pink	reflexed	N, P, K, Ca, Mg, Mn, B	N, P, K, Ca, Mg, Mn, B	B
4	Sussex Bronze	bronze	reflexed	—	N, P, K, Ca, Mg, Mn, B	Mn, B
5	Sussex Red	red	reflexed	—	N, P, K, Ca, Mg, Mn, B	B
6	Sussex Pink	pink	reflexed	—	N, P, K, Ca, Mg, Mn, B	Mn, B
7	Market Gold	golden yellow	reflexed	—	N, P, K, Ca, Mg, Mn, B	K
8	Autocrat	white	semi-incurved	—	N, P, K, Ca, Mg, Mn, B	K, Mn, B
9	Monument	white	incurved	—	—	Mn, B
10	Friendly Rival	golden yellow	incurved	—	—	B
11	Edith Alston	white	reflexed	—	—	B
12	Edith Alston	white	incurved	—	—	B
13	Blazing Gold	golden yellow	incurved	—	—	B
14	Penrod	golden yellow	incurved	—	—	B
15	Annette	white	reflexed	—	—	B

RESULTS

No Nitrogen

The first indications of this deficiency were apparent about a month after the treatment began and consisted of a loss of colour in the older leaves and a reduction in size of the younger ones. As the younger leaves developed they assumed an erect position and acquired a pale colour. Later the older leaves became more yellow with pigmentation of the margins in some varieties — namely Sussex Bronze, Sussex Red, Imperial Pink and Market Gold. The whole range of colours from slightly yellowish green at the top, yellow green in the middle to yellow and yellow with red

pigment in the oldest leaves could be seen together on any one plant. The lowest leaves turned brown and died prematurely. The plant survived till flowering time but only a few of the uppermost leaves retained any green colour and were turgid at that time. The lateral buds remained dormant. Soon all growth stopped and stems became woody. A reduction in the rate of growth occurs very early and this is the first indication of this deficiency. Opening of flowers is delayed for from 3 to 5 weeks. The diameter of the open bloom is reduced to just over half of that of the controls. The effect on colour varies there being a general tendency to produce more intensely coloured blooms especially on red and bronze varieties; no effect was observed on the blooms of any of the pink varieties used. In all the varieties the petals were firm and the keeping quality was better than in any other treatment, including control.

There is very little difference in the quality of the response of different varieties to this treatment. Some, like Rose Harrison and Imperial Pink, showed the first symptoms within 3 or 4 weeks while others, like Sussex Bronze and Sussex Red developed them in 5 or 6 weeks, the remainder being intermediate. Although it is probable that these differences were due to varietal susceptibility connected with the habit of growth it cannot be ruled out that the poorer root system of cuttings of the Sussex varieties at the time of transferring to sand in 1949 and subsequent slower growth of those varieties may have been partly responsible. It is interesting to note that all the varieties show the same general sequences in the development of symptoms irrespective of the time taken for them to appear.

There are two other varietal differences worth mentioning. First the purplish-red pigment on old yellowing leaves, referred to earlier, which was abundant on some varieties was absent on Bronze Harrison and Autocrat. Rose Harrison and Sussex Pink developed it occasionally but never to the same extent as the first group.

The second is the way the leaves die at the later stages, when the deficiency becomes very acute at about the time the buds are formed. In Autocrat at this stage a distortion of the leaves was noticed which almost amounted to puckering. It can be best described by supposing that thread is inserted round the edges of

the leaf and then pulled slightly. This distortion is closely followed by browning and dying of tissue in a narrow belt at a certain distance from the edges. This phenomenon explains the earlier distortion. On all other varieties bronzing and dying of tissue occurred on the actual edges and no earlier distortion was observed. In both cases death of the whole leaf followed quickly.

No Phosphorus

Here again the effect is general, the whole plant being affected. The rate of growth is reduced, the young leaves are small and set at an acute angle; internodes are not shortened and growth is spindly. This last symptom together with the fact that there is no general loss of colour makes it easily distinguishable from shortage of nitrogen. At a later stage the whole plant is of a dull green colour.

The yellowing, browning and drying out of old leaves are the next symptoms. These stages succeed one another closely so that at no one time are there more than a few yellow leaves on any one plant. Purplish-red pigments appear on some varieties. The dead leaves are darker brown than those on no nitrogen plants. Here again lateral buds are dormant.

The treatment retards the opening of the flower buds and causes a reduction in the size of the blooms. The flowers are usually slightly paler. There was no noticeable effect on the actual texture of the petals.

Rose Harrison and Sussex Pink are different from the other varieties studied in two respects. First, when the older leaves begin to die this proceeds rapidly, whereas in the other varieties this happens comparatively slowly. Secondly, they show no regularity in development of symptoms on individual leaves; in the other varieties there is a regular sequence in that effects are first seen at the apex of the terminal lobe and they then develop along the midrib.

No Potassium

Wide differences were observed in the manner potash deficiency manifests itself. Symptoms differ quite appreciably from season to season and from variety to variety. The age of the plant at the time the deficiency begins to operate is probably the most im-

portant single factor affecting the appearance of symptoms and the general condition of the whole plant.

The first symptoms are noticed on the middle leaves and consist of more or less regular marginal chlorosis changing rapidly into marginal scorch. The two follow each other so closely that the first may easily be overlooked. Rate of growth becomes slower, internodes are short, and the size of the leaves is greatly reduced. The leaves which were first affected die and the older leaves die at an abnormal rate. At a certain stage symptoms are very similar to those caused by eelworm attack. Interveinal chlorosis of the youngest leaves is quite a common symptom at the later stages. It is interesting to note that in varieties such as Autocrat which show the regular maginal scorch the young leaves are not only much reduced in size but are completely chlorotic including even the veins. On the other hand those with irregular first symptoms such as Sussex Bronze and Imperial Pink show less reduction in size and only marginal and interveinal chlorosis.

A pronounced tendency to produce basal cuttings and a poor root system are other characteristic symptoms.

The quality and colour of the blooms were not noticeably affected, but the size was invariably reduced. There was some tendency for all the blooms to open earlier in all varieties used.

All the foregoing effects are much less clearly defined when well established plants are deprived of potash. Under these conditions all varieties show some reduction of growth, rather irregular dying of the middle and older leaves, and excessive growth of basal shoots. The most noticeable symptom is, however, comparatively poor development of roots and marginal and interveinal chlorosis of young leaves at the end of the season. The two are most probably related and as suggested by many workers dealing with other subjects poor root development may lead to iron deficiency of which the chlorosis is reminiscent.

Where young plants are used the effect on growth differs from variety to variety. A good example of this is the sudden arrest of all growth in Imperial Pink within three weeks as compared with Sussex Bronze in which six weeks were required to reduce the growth rate. The differences in pattern of the first symptoms on leaves is well illustrated on Plate I. Of those three varieties only Autocrat shows regular marginal scorch. In Sussex Bronze necrosis

occurs in various parts of the leaf, tip scorch being most prevalent. In Imperial Pink, however, although regularity is not so marked the end nearer the petiole is usually first affected. It was also noted that some varieties, of which Market Gold is an outstanding example, show a very definite distortion of shoots as the results of shortage of potash. The tendency is for shoots to spread away from the vertical much more than in plants grown on complete nutrient solution (see Plate IIA and B). Autocrat is another variety which exhibits this tendency but only when the deficiency operates from the early stages of growth.

No Calcium

Of the effects due to deficiencies those which follow the omission of calcium take the least time to manifest themselves. Depending on the variety, the season and the state of development of the plant at the time treatment was started effects were observed between the 16th and the 28th day. The youngest leaves become severely chlorotic; in a few days brown spots, irregular in shape and scattered mostly along the margins appear. Soon the whole leaf margin becomes bronzed and the distortion of the leaf which curled downwards is evident. Death of the growing point soon follows and slow dying back of the stem takes place. In most cases lower leaves continue to grow and survive till the end of the season, being sometimes even larger than corresponding ones on the control plants. In most varieties these leaves were dull dark green in colour, distorted by curling downwards and inwards, stiff and brittle, with occasional irregular chlorotic areas.

When the treatment was started later and the death of the growing tip did not take place before bud formation, the most characteristic symptom was the acute shortening of the internodes on the flower stem, which rarely exceeded 3 inches in length, as compared with 2 to 3 feet on control plants. The buds always died at an early stage of development and the leaves remained very small and chlorotic.

The roots were badly affected, the main ones being short and stunted with signs of considerable dying back at the tips. The fine roots were dead and blackish-brown in colour.

All varieties reacted in a similar manner. Rose Harrison is most susceptible, symptoms appearing earlier and the effect being more pronounced.

Plate I. Varietal differences in potassium deficiency symptoms. A. Autocrat showing marginal scorch. B. Imperial Pink showing the base of the leaf affected first. C. Sussex Bronze showing irregular necrosis with pronounced tip scorch.

A B

C

Plate II. A and B Potassium deficiency in Market Gold. Leaves removed from both plants. A. Spreading of shoots in affected plant B. Control plant. C. Manganese deficiency symptoms on middle leaves of Rose Harrison. Leaf on the right was pressed flat to show the pattern. Centre leaf from control plant.

There are also differences between varieties in the intensity of chlorosis in the youngest leaves and the appearance of spots following it. On Imperial Pink, Sussex Bronze and Sussex Red leaves become nearly yellowish-white and only a few very small spots appear before the leaf dies completely. On the other hand on Rose Harrison, Autocrat and Sussex Pink chlorosis is not so acute, large and definite brown spots develop along the edges of the leaf. In the youngest leaves these spots coalesce into marginal scorch.

No Magnesium

Here the effects differ widely in the different varieties. Growth generally was but little affected when well established plants were subjected to the treatment and only slight loss of colour had occurred by the end of August. On the other hand severe symptoms appeared within a short time when young plants were used. The internodes were shortened and no flowers were produced. Even in the former case three different types of symptoms had developed in the foliage by November.

1. Regular interveinal chlorosis was general and this was followed by the development of purplish-red tints. The condition is first observed on old and middle leaves which eventually become bronze-coloured and die. Sussex Red and Sussex Bronze conform to this behaviour. In the case of Market Gold the condition was much more pronounced, but no exact comparison is possible as this variety was not represented in the "late started" series. In the "early" series there was practically no growth of the flower stems and the small buds died without opening.

2. General loss of colour in the foliage followed by the older leaves turning yellow. Irregular bronze-coloured areas appeared quickly and these soon became desiccated. Rose Harrison and Sussex Pink fall into this category and the latter occasionally developed purplish-red tints.

3. A barely perceptible loss of colour took place in the old leaves, some scattered areas of purplish-red colouration appearing on only a few of the older ones. Then in a very short time, the whole of the interveinal areas of both old and middle leaves became covered with small circular brown spots. These spots do not extend but gradually darken in colour and dry out. Imperial Pink behaved in this way.

Autocrat does not conform to any of the three categories. In this variety there was a limited loss of colour in the foliage, some marginal scorch of old leaves and a loss of colour round the edges of the youngest leaves when the flower buds were opening.

The sudden and very quick development of the symptoms is very characteristic of magnesium deficiency. When well established plants are used symptoms appear late but develop rapidly. This is most noticeable on Rose Harrison and Sussex Pink. Practically the whole of the foliage is affected by the time the blooms are fully open.

In the case of young plants symptoms appear early and further growth is completely arrested.

The flower buds opened comparatively early on all varieties. The size of blooms was reduced, particularly in Autocrat, and colour was paler in all coloured varieties, Rose Harrison being most affected in this respect.

No Manganese

Lack of manganese in the nutrient solution results in the appearance of very characteristic symptoms in the foliage; the vigour of the plants is greatly reduced and last but not least, the flowers are of indifferent quality, their petals lacking crispness and they do not keep well.

Here again results may differ from season to season. Young plants react to the treatment much more quickly and the effects are very acute as compared with those on well established plants. Varietal differences in susceptibility to this deficiency are clearly marked. The pattern of symptoms varies considerably.

In most cases the first symptoms are the marginal and interveinal chlorosis on the middle leaves. The poor vigour of the plant is indicated by thin, spindly shoots and smaller leaves. Some general loss of colour is also noticeable. Later the middle leaves curl downwards at the edges and tips in a characteristic manner. Concurrently these leaves develop reddish-brown areas within the chlorotic parts. These soon dry out.

The fine notting effect on the young leaves, so often described as a characteristic symptom of manganese deficiency, was observed on most varieties. In more acute cases it is followed by necrosis in the form of small roundish light-brown spots. The varieties,

however, differ appreciably in their tendency to produce this effect and the influence of the season appears to be greater on this particular symptom than on others.

Flowering is delayed and the size, colour and texture of the blooms are all affected.

Rose Harrison appears to be more susceptible than any other variety. The first symptoms were noted much earlier, especially in 1949, and were more acute. Very acute interveinal chlorosis develops on the middle leaves. Before the necrosis sets in, rather bright brownish-red tints are produced, and characteristic curling downwards at the edges and tips occurs (see Plate IIC).

The same pattern is shown by Sussex Pink, but symptoms appear some 3 or 4 weeks later, and the effect on blooms is less pronounced. Both varieties develop fine netting on the young leaves.

The other two susceptible varieties are Monument and Autocrat. The first symptoms appear early in Monument but the effect on growth is not very pronounced. Chlorosis on the middle leaves appeared almost at the same time as in Rose Harrison. Necrosis developed more slowly and although finally it is as pronounced as on the other variety the leaves remain flat and no tints were observed. By the end of the season young leaves show well defined netting. Autocrat is slower in showing the first signs of disorder, but once they start symptoms develop quickly; growth is stunted and blooms are extremely poor. Marginal chlorosis spreads rapidly from the middle leaves to those at the top but no netting effect appears on them.

Imperial Pink shows slight general loss of colour throughout the plant and interveinal chlorosis with some necrosis on the top leaves, which sets in in the form of small roundish spots, dark brown in colour.

Sussex Red and Bronze were least affected and marginal chlorosis on the middle leaves was the only visual symptom. Stems were thinner and the quality of the blooms was inferior especially in Sussex Bronze.

No Boron

The first signs are invariably shown on the young leaves which become chlorotic. On some varieties whole leaves turn yellow

and acquire an erect habit. Internodes are extremely short, new leaves are very small and they are the first to die. The death of the growing tip is followed by dieback of the stem. In less susceptible varieties chlorosis is interveinal and shortening of the internodes less acute. In the "late" treatment the death of the growing tip did not occur and noticeable recovery took place during the shorter autumn days.

With treatment adjusted so that the omission becomes operative when all the leaves have been formed the main effect is that leaves show considerable distortion and they curl downwards, the tip coming close to the petiole (see Plate IIIB). Leaves may grow to an appreciable size but are very brittle so that they break off from the stem very easily. The veins become brown and crack at various points. Irregular interveinal chlorosis occurs and purplish-red tints develop on some varieties.

Root systems under this treatment was examined at the end of the season and the effect was similar to that described for "no calcium" treatment.

The two varieties Monument and Autocrat are most affected and react in the same way. The colour of the young leaves is only slightly affected and internodes are extremely shortened. Leaves curl downwards and are very brittle. Irregular chlorotic areas appear and the veins crack. Blooms were not produced even with "late" treatment (see Plate IVC). Rose Harrison comes next so far as susceptibility is concerned, the first symptoms appearing even earlier than on the two previous varieties. At least 40% of the shoots produced fail to produce blooms. It was the only variety which developed brown cavities in the stems, and one of the two, the other one was Autocrat, to show some effect on blooms when demineralised water was used for preparing nutrient solution. Symptoms on the foliage are most striking, young leaves becoming completely bleached or greatly distorted depending on the time the treatment starts (see Plate IIIA).

The effects on the foliage of both reflexed and incurved Edith Alston are not pronounced, chlorosis being interveinal. The shortening of internodes is less acute, particularly on the incurved variety, and distortion of leaves and cracking of veins occurred only at the end of the season.

The two varieties which showed practically no symptoms on the

A

B C

Plate III. Boron deficiency symptoms. A. Rose Harrison - acute chlorosis on uppermost leaves. B. Edith Alston (reflexed) - characteristic distortion of leaves. C. Petals of Edith Alston (reflexed). *Left:* Affected petal showing lack of sheen and smoothness *Right*: control petal.

Plate IV. Effects of boron deficiency. A. Annette. B. Sussex Bronze C. Monument. Control blooms on the left.

foliage are Blazing Gold and Friendly Rival, but as these were kept out of doors through the summer the results are not strictly comparable with those for other varieties. Both however exhibit the compactness of the petals and their texture was adversely affected.

All other varieties show interveinal chlorosis on the youngest leaves and this spreads slowely down the plant. Slight shortening of internodes also occurs and the stems are weaker. Considerable recovery is noted when the days become shorter. In all cases the blooms were affected. It appears that as a group the white varieties are more affected by this deficiency than others. It is of interest in this connection that Laurie and Kiplinger[14]) found that some white varieties of roses benefit from extra boron in nutrient solution (up to 5 p.p.m.). On the other hand they state that boron poisoning of roses may occur at as low a concentration as 1 p.p.m.

Effect on blooms. The effect of this treatment on the blooms is perhaps the most striking outcome of this work. In all varieties some distortion of the ray florets was produced. The first noticeable sign was a lateral rolling of the florets to give almost a tubular effect. This was followed by twisting of the outermost florets. The next stage was curling upwards and inwards longitudinally thus producing incurved blooms on varieties which are normally reflexed (see Plate IVA and B). The magnitude of these effects is not the same on all the varieties used. In Annette, Edith Alston (reflexed), Sussex Bronze, Market Gold and Imperial Pink the florets are rolled laterally and the incurving is very pronounced. The size of the blooms is considerably reduced. In Sussex Red there was little rolling, but incurving was marked. This would appear to be similar to that reported by Ferguson and Wright[6]) working with the variety Marie Adelaide.

In Rose Harrison and Sussex Pink there was definite lateral rolling but only slight incurving. Here the size of the blooms was not so seriously effected.

All the coloured varieties showed some loss of colour as the result of the treatment.

All incurved varieties showed considerable "tightening" of the blooms.

Close examination of the individual petals show that their shapes

and conformation are seriously affected. All the varieties show these symptoms.

As an example the effects on the petals of Edith Alston reflexed are described in some detail.

The petals of this variety grown normally are more elaborate than those of many other varieties. In particular the tubular part at the base of the petal is quite distinct which is somewhat uncommon. Ordinarily the tubular part leads to a funnel-shaped lobe which may be up to 5 mm in length. Where boron is omitted the "funnel" is reduced in length or may be non-existent and the tube also is considerably shortened. The texture of the florets is appreciably altered. Instead of the sheen and the smoothness of petals of normally nourished plants the surfaces become dull and rough almost to the point of irregular corrugation (see Plate IIIC).

Another striking feature is a high susceptibility to bruising. Normal petals turn grey and tend to become transparent at the point of contact. In the absence of boron, however, the bruised part becomes brown in a matter of minutes. This is no doubt related to the enzyme status of the plant and it is hoped to investigate this later. Individual petals tend to curl upwards and inwards so that the overall effect on the bloom as a whole is to make it perfectly incurved. The loss of the sheen and smoothness was observed on all varieties. The effect on the shape naturally varies a great deal, the extent depending on the shape of normally nourished petals; for example on incurved varieties the blooms are tighter in the absence of boron but the shape of petals is hardly altered.

DISCUSSION

The results of this work have shown that as with other species different varieties of chrysanthemums react differently to the same acute mineral deficiencies.

Symptoms differ considerably and it appears that while one variety may fail completely to grow, or at least to produce flowers another may under the same conditions show only slight signs of abnormality. It can also be said that even the detailed knowledge of the visual symptoms on one variety may not be sufficient to identify the same disorder on others and that it is necessary to work with representative samples of the varieties before attempting to describe the visual symptoms for the species.

Further complications are introduced by the seasonal variations and the age of the plants at the onset of the deficiency. That is specially true in case of trace element deficiencies where the reactions vary considerably depending on the climatic conditions during the season. Among the major elements symptoms of potash deficiency are more dependent than any others on the weather conditions and the stage of growth of the plant.

Magnesium deficiency symptoms develop very early in young plants and its lack at this stage stops all growth and kills the plant. On the other hand with fully developed plants the symptoms appear late and the effect on growth is slight. Even then, however, once symptoms are evident in the leaves they develop rapidly.

Hill *et al.* [12]) say "there is some indication that response may be qualified in degree and character by variety" and that "it may be necessary to group varieties according to their nutritional requirements." The present investigations confirm both statements and we would suggest further that for the purpose of recognising the visual symptoms of malnutrition there is a possibility of linking the growth characteristics of the variety, foliage character, the colour of the blooms etc. with the way the effects will manifest themselves. This may be of great importance, especially in the case of a crop such as chrysanthemums of which there are almost innumerable varieties.

No Nitrogen

In general the effects now described agree with those described by other workers and are much the same as for many other species. Chrysanthemums do not appear to be extremely susceptible to this deficiency and they survive to maturity better than some other plants. In this connection it is interesting to mention the work of Sen [20]) who concludes that leaves of higher plants under starvation conditions may develop the ability of assimilating nitrogen from the atmosphere. In spite of the death of most of the foliage they produce flowers of good quality although these are severely reduced in size. The deeper colour of the blooms was mentioned by Hill *et al.* [12]) and was noted in more than one variety in our experiments. The distortion of the leaves, and the narrow belt of dying tissue produced on them at the time of opening of the blooms in Autocrat, is noteworthy, especially when considered

in conjunction with marginal chlorosis on the young leaves on no-potassium and no-magnesium treatments exhibited at about the same stage by this variety. It appears that the formation of the flower involves a greater strain on the plant in this variety than in any other. Cöic [5]) reports marked varietal differences in the nitrogen requirement of wheat, particularly in the later stages of growth.

The delay in flowering, which we have reported in nitrogen starved plants, is in agreement with the findings of Chailakhyan [3]) and many other workers referred to in the review of the subject in Truog's book [23]).

No Phosphorus

The symptoms resemble very much those on other species. The susceptibility appears to be comparatively high. The leaf symptoms agree with the description by Hill *et al.* [12]) rather than with that by Laurie and Wagner [15]). In no case was dropping of leaves observed as is suggested by the latter workers. When dead, the leaves hang on the stems as in other deficiencies, but they are of a noticeably darker brown colour.

Although considering it only in relation to potassium status Hill *et al.* [12]) found increased intensity of colour of the blooms with phosphorus deficiency. This was not observed in the present work.

No Potassium

The typical marginal scorch shown by most species is shown on some varieties. The great variability of the symptoms is in agreement with other investigations on a wide range of subjects. The variations in the symptoms shown by different varieties of chrysanthemums described here are strikingly similar to those found by Lineberry and Burkhart [16]) working with two varieties of strawberries.

The susceptibility of the different varieties varies greatly and is accentuated when young plants are deprived of potash. Hill *et al.* [12]) noted it for the two varieties of chrysanthemums used in their work.

The distortion of shoots observed in the present work does not appear in the literature on chrysanthemum nutrition. The tendency

to produce basal shoots is undoubtedly the same phenomenon as tillering of cereals which as Gregory[9]) states is stimulated by a shortage of potassium when nitrogen and phosphorus levels remain high. Hewitt[10]) also reports increased tillering of cereals when potassium is deficient.

The chlorosis of the young leaves, very similar to that described as due to iron deficiency, may in fact be due to the inability of the plant to take up sufficient amount of that element as the result of the poor root system. The alternative explanation is suggested by Wallace and Hewitt[26]) who present evidence suggesting some association between potassium status and iron mobility within the plant.

No Calcium.

The symptoms followed a pattern described for many species. The layout of the experiment does not allow definite classification of this crop as calciphilic or calciphobic but in general, susceptibility to calcium deficiency appears to be great and death of the growing point occurs fairly quickly. Our results agree with the description given by Laurie and Wagner[15]). The early effect on the roots is characteristic.

No Magnesium

The great variability of the visual symptoms of magnesium deficiency has been stressed by many investigators working with various plants. Wallace[25]) found that two varieties of apple trees show a dissimilar pattern. There is a concensus of opinion that the appearance of the first symptoms is delayed when fully developed plants are deprived of this element, but once they appear rapid development usually occurs. Both these points were amply confirmed in the present study. In fact variations exhibited by the different varieties of chrysanthemums are quite often greater than those between the unrelated species. In the main our results agree with Laurie and Wagner[15]). Exceptions are first, in the present work blooms opened earlier or at the same time as those on control plants. The only case where their formation was delayed was in "early treatment," in which complete death of shoots occurred in most cases before the buds opened. Secondly the effect on roots was comparatively small. Thirdly the upward curling of the leaf

described by these authors was not noted in our experiments; "buckling" or downward curling appeared on some varieties. These differences may be due to the different varieties used and also to the fact that Laurie and Wagner[15]) apparently started the treatments very early in the life of the plant.

No Manganese

With slight seasonal variations the symptoms appeared quickly and the effect was pronounced. There exist appreciable differences in susceptibility between the varieties. The general pattern agrees with that described for many other species.

Our results are in close agreement with those of Laurie and Wagner[15]) reported for the variety Valencia, especially when the two more susceptible varieties Rose Harrison and Sussex Pink are considered. The marginal chlorosis on the middle leaves was the first symptom and then curling of those leaves was observed. The chlorosis of the uppermost leaves producing the fine netting effect developed later. In the case of less susceptible varieties the growth was practically unaffected and the effect on young leaves, late in the season, was the only sign of disorder. In all the more acute cases the middle leaves were invariably first affected and it is probable that so far as chrysanthemums are concerned this symptom is as important as the netting usually associated with manganese deficiency. It appears early on susceptible varieties and unless the condition is corrected they produce weak shoots and blooms of very poor quality.

No Boron

A very quick reaction followed the withholding of this element. The first signs appeared in 3 -4 weeks in the case of well established plants. When operations were adjusted to enable the blooms to develop, very interesting effects on the shape and texture of petals were noted. They confirm the observation of Ferguson and Wright[6]) on curling of the petals on Marie Adelaide, which is the only record of boron deficiency symptoms in chrysanthemums we have been able to trace.

In general the symptoms noted are in good agreement with those described for many plants. Some recovery during short day periods was invariably noticed.

SUMMARY

The visual symptoms of the deficiencies of nitrogen, phosphorus, potash, calcium, magnesium, manganese and boron on indoor chrysanthemums are described in detail and illustrated. Fifteen varieties were grown in sand cultures and their behaviour studied. The importance of varietal susceptibility and effect of season are established.

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REFERENCES

1) The American Society of Agronomy and the National Fertiliser Association (Publisher) Washington. — Hunger Signs in Crops (symposium) 1941.
2) Brooks, J. H., The effect of a deficiency of nitrogen, phosphorus and potassium upon the growth of chrysanthemums (abstract). Proc. Am. Soc. Hort. Sci **29**, 544 (1932).
3) Chailakhyan, M. Kh., Flowering in different plant species as a response to nitrogenous food. Compt. rend. acad. sci. U.R.S.S. **47**, 146–149 (1945).
4) Chilean Nitrate Educational Bureau, Inc. (Publisher) New York 5, N.Y. — Bibliography of the literature on the Minor Elements and their Relation to Plant and Animal Nutrition, **1** (1948) and **2** (1951).
5) Coic, Y., La nutrition azotée du blé. Ann. Agron. **3**, 417–421 (1952).
6) Ferguson, W. and Wright, L. E., Micro-element studies with special reference to the element Boron, Sci. Agr. **20**, 470–487 (1940).
7) Gericke, S., The action of the nutrient substance, phosphoric acid, in chrysanthemum culture. Chem. Zentr. **II**, 1622 (1943).
8) Goodwin-Wilson, R., Analysis of horticultural soils, Sci. Agr. **25**, 175–178 (1944).
9) Gregory, F. G., Mineral nutrition of plants. Ann. Rev. of Biochem. **6**, 557–578 (1937).
10) Hewitt, E. J., Experiments in mineral nutrition II. The visual symptoms of mineral deficiencies in crop plants grown in sand cultures. Long Ashton Research Sta. Ann. Rep. 1944.
11) Hewitt, E. J., Use of water purified by synthetic resin ion-exchange methods for the study of mineral deficiencies in plants. Nature **158**, 623 (1946).
12) Hill, H., Davis, M. B. and Johnson, F. B., Nutritional studies with chrysanthemums, Sci. Agr. **15**, 110–125 (1934).
13) Krawczynski, St., Orientative studies on the fertilization of "Miss Edith Cavell" and "Mme Rene Oberthur" two varieties of chrysanthemum indicum L. Uprawa Róslin i Nawozenie **9**, 81–82 (1939).
14) Laurie, A. and Kiplinger, D. C., Commercial Flower Forcing. The Blakiston Company, Philadelphia–Toronto. Fifth Edition (1948).
15) Laurie, A. and Wagner, A., Deficiency in greenhouse flowering crops. Ohio Agr. Exp. Sta. Bull. 611 (1940).
16) Lineberry, R. A. and Burkhart, L., Nutrient deficiencies in the strawberry leaf and fruit. Plant Physiol **18**, 324–333 (1943).
17) Mazaeva, M. M., Sensativity to Magnesium Deficiency and Photoperiodic Reaction in Plants, Compt. rend. Acad. Sci. U.R.S.S. **73**, 191–194 (1950).
18) Owen, O., The Effect of Manurial Treatment on Nitrogen Contents of some Market Garden Crops. Rep. Exp. Research Sta. Cheshunt 1933.

19) Owen, O., The Effect of Manurial Treatment on the Mineral Constituents of Chrysanthemums. Rep. Exp. Research Sta. Cheshunt 1934.
20) Sen, S. C., Some aspects of nitrogen fixation by higher plants. J. Proc. Inst. Chemists (India) **24**, 33–38 (1952).
21) Stecher, H., Effect of graded additions of nutrients on the quality and nutritional balance of chrysanthemum indicum. Chimie et Industrie **47**, 272 (1942).
22) Stiles, W., Trace Elements in Plants and Animals, Cambridge University Press (1944).
23) Truog, E., (editor) Mineral Nutrition of Plants. The University of Wisconsin Press 1951.
24) Wallace, T., The Diagnosis of Mineral Deficiencies in Plant by Visual Symptoms, H.M.S.O., London, 1951.
25) Wallace, T., Magnesium-Deficiency of Fruit Trees. J. Pomology and Hort. Sci. **17**, 150–166 (1939).
26) Wallace, T. and Hewitt, E. J., Studies in iron deficiency of crops. I. Problems of iron deficiency and the interrelationships of mineral elements in iron nutrition. J. Pomology and Hort. Sci. **22**, 153–161 (1946).

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INVESTIGATIONS CONCERNING NODULATION AND GROWTH OF LUCERNE SEEDLINGS IN A LOESS SOIL ARTIFICIALLY AGGREGATED TO VARIOUS LEVELS

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Following the experiments made by Hanotiaux and G. Manil[1]) and Hely and Bonnier[2]) on the effect of some synthetic resins, developed for the amelioration of soil structure, on soil aggregation and on the associated population of gum-producing bacteria, further studies were made which involved the plant lucerne. Although the loess-derived soils of the Gembloux region are in a good enough state of fertility physically and chemically to allow of the development of what might be considered to be normal nodulation of lucerne derived from inoculated seed, it was thought to be desirable to examine the effects of such chemically produced aggregation on the nodulation and general growth of lucerne seedlings.

1. SOIL MATERIALS AND AGGREGATING SUBSTANCES USED

To do this three pot experiments were set up and conducted under controlled glasshouse conditions. In two of them the soil used was the randomly sampled topsoil from a highly productive cultivated field which had first grown a heavy crop of wheat and in which the level of available nitrogen was at a minimum; it had a pH of 7.8. For the other experiment the corresponding loess parental material located at a depth of 1.5 m to 2.0 m was used; this had only a very small amount of total humus, a trace of available nitrogen and no macro-aggregates; its pH was approximately 6.0 but this was brought back to neutral by treatment with precipitated $CaCO_3$ well in advance of potting time.

These soils were taken by random sampling, put through a 3 mm screen while they were still somewhat moist and then air-dried and thoroughly mixed in each case. For each one the level of available P_2O_5 was brought up to 200 mg per 100 g of soil and the level of available K_2O was brought to 100 mg per 100 g of soil.

Mechanical analyses were made on these mixed samples at the Pedology Laboratory of this Institute and the results are shown in Table I. There was a small amount of colluvial or detrital material mixed with the top soil.

TABLE I

Fractions	Clay	Silt		Very fine sand	Fine sand	Gross sand
Particle size in microns . . .	0–2	2–10	10–20	20–50	50–100	100–3000
Top soil. . . .	13.95	11.89	20.15	47.44	3.61	2.97
Parent material	20.10	5.64	15.20	52.15	6.66	0.26

The desired amounts of these soils were treated with the appropriate amounts of the polyelectrolytes in solution by spreading each of the air-dried soil lots thinly and then watering on as uniformly as practicable the resin dissolved in just enough water to wet all of the soil to the moist crumbly stage; the soils were raked and mixed as they were being wet. After it had dried out somewhat, each lot of soil was again put through the 3 mm screen while still a little moist and then air-dried before being weighed into the pots.

These aggregating substances were: (1) the product "Aglusol", a solution of 5 per cent of active principle which was supplied by the Union Chimique Belge and contained an appreciable amount of ammonia residual from the process of manufacture, (2) "Aglusol" from which the ammonia had been removed, (3) a similar product supplied by the Union Chimique Belge which was free from ammonia and known as SRS 88/52 and (4) the original form of "Krilium" as furnished by the "Monsanto" firm; the latter was the hydrolysed polyacrylonitrile in crystalline form.

The surface soil was very responsive to treatment by these substances but the loess parental material was rather less responsive. To get a reasonable measurement of the effect of these soil conditioners on the soil materials employed determinations were

made on the percentages of water-stable aggregates larger than 0.5 mm in diameter; these macro-aggregates were considered by Martin[3] and others to be of greatest practical significance.

To determine the percentage of water-stable aggregates present at various times in the untreated and treated soil lots a modification of the Yoder[4] wet sieving technique was used. In each case two separate 50 g samples of soil were taken by random sampling and analysed for water stable aggregates (1) greater than 1.0 mm in diameter and (2) between 0.5 mm and 1.0 mm in diameter. In most cases very close agreement was obtained between the two replicates; where it was not so, more 50 g samples were analysed to meet the needs of the situation. To minimize the breaking of aggregates by the air escaping explosively from within them, such as may happen when dry soil is wet rapidly, all soil samples were wet slowly and carefully with atomized distilled water. These lots then were submerged in distilled water and soaked for 24 hours to test the capacity of the soil aggregates present to withstand the slaking effects of water. Subsequently the samples were washed carefully from the beakers in which they had been soaked and screened out carefully under water using only the 1.0 mm and 0.5 mm mesh screens. The aggregates obtained on each screen were washed off on to filter paper, dried at 103° C and weighed. To allow for small amounts of sand, etc. included with aggregates these samples were treated subsequently with a sodium salt dispersing agent and screened again under water to remove all finer particles; appropriate correction was made by subtracting the dry weight of such residual coarser material from the gross weight of the aggregates plus sand, etc.

2. DESIGN OF EXPERIMENTS

In the first experiment large, glazed, cylindrical earthenware pots capable of holding readily 11 kg of the untreated top soil were used. Top soil was treated at the three levels of 0.01 per cent, 0.05 per cent and 0.10 per cent in the case of each of the four substances mentioned above. To compensate for the increase in soil volume brought about by aggregation with the greater quantities of the products, a compromise was made by putting a little less soil than 11 kg in the control pots so that nearly the same weight of soil could be put into the more completely filled pots

containing the 0.10 per cent treatments. A layer of clean gravel was placed in the bottom of each pot and a glass tube was inserted to allow for the application of water from below. The soil was compacted in each pot by an unmeasured but fairly uniform amount of bumping down and pressing. A calculated amount of water equal to 80 per cent of the field capacity was added and maintained by subsequent additions with weighing.

It was practicable to have only two replications but there were double controls and the pots within each group were randomized anew and re-arranged once a week, so as to even out differences in the environmental conditions in the glasshouse.

Seeds of Provence lucerne were selected carefully for large size, healthy appearance and uniformity, then surface sterilized with ethyl alcohol and a solution of mercuric chloride 0.25%, and finally washed and set to germinate asceptically on yeast-mannitol agar plates at 20°C. From these plates two days later vigorous, healthy seedlings were chosen, inoculated with the widely used, superior and effective S. 22 strain of *Rhizobium meliloti* and planted carefully in these pots. In each pot 30 such seedlings were placed and then, as soon as their establishment was certain, the number per pot was reduced to 25, the apparently best plants being retained. Some of the earlier waterings were applied gently from above by watering can to help consolidate the soil.

The second experiment was in every respect identical with the first except that the loess parental material was used rather than the surface soil and this was denser so that the pots would hold 12 kg of untreated soil material.

In the third experiment the surface soil was employed again and somewhat smaller and unglazed pots were used. Only the levels of 0.05 per cent and 0.10 per cent were used for each of the four products but eight replications were prepared. Here again the randomization was re-arranged each week. The main differences between this and the first experiment were that in this a dense stand of the same lucerne was used by sowing selected, inoculated seed directly into the pot so as to obtain approximately ten times as many seedlings per unit area as were present in the large pots. Also in this case all watering was done from above by watering can and in such a way as to simulate fairly heavy rainfall as nearly as practicable.

3. RESULTS OBTAINED

Since the percentage of water-stable aggregates is a measurable characteristic which serves as a useful indicator of soil structure, attention was paid to this aspect with a view to the interpretation of any plant differences which might be observed.

In Table II are shown the percentages of water-stable aggregates greater than 0.5 mm in diameter which were found in the soil lots at the beginning and end, after three months, of the first experiment. This, of course, was carried out with surface soil.

TABLE II

Soil Treatment	Percentage of water-stable aggregates in surface soil					
	Commencement of Experiment			Conclusion of Experiment		
	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm
None	16.2	4.4	20.6	6.5	2.5	9.0
0.01% Aglusol	20.3	13.5	33.8	7.2	4.9	12.1
0.05% „	16.3	26.1	42.4	12.0	25.4	37.4
0.10% „	15.2	50.6	65.8	10.4	62.7	73.1
0.01% Ammonia-free Aglusol	18.0	10.8	28.8	4.4	2.8	7.2
0.05% Ammonia-free Aglusol	8.3	45.9	54.2	11.4	24.0	35.4
0.10% Ammonia-free Aglusol	8.7	54.8	63.5	14.8	41.1	55.9
0.01% SRS 88/52	11.9	12.3	24.2	10.8	8.5	19.3
0.05% SRS 88/52	15.1	32.0	47.1	13.3	31.7	45.0
0.10% SRS 88/52	12.8	51.7	64.5	12.9	43.4	56.3
0.01% Krilium *)	16.1	4.5	20.6	2.3	1.1	3.4
0.05% „	13.7	12.5	26.2	7.5	6.4	13.9
0.10% „	11.6	22.1	33.7	10.4	15.8	26.2

*) 83% of active principle.

In these large pots the soil mass was not well occupied by lucerne roots and therefore presumably the influence of possible gum-producing rhizosphere organisms was not important. Consequently there was in general a net loss in water-stable aggregates greater than 0.5 mm in diameter. This break-down of macro-aggregates occurred much more obviously where the low concentrations of the products had been used. Where the high concentrations had been employed on this soil the aggregates were stronger and more persistent besides being more numerous; and furthermore there was

in these cases a significantly greater proportion of the larger aggregates as compared with the smaller. There was a marked tendency for this situation to be reversed where the low concentrations of the products had been used. The soil treated with the 0.1 per cent natural "Aglusol" was exceptional but the indications were that the additional ammonia received by this treatment had stimulated additional microbial activities and resulted in the binding of smaller aggregates into larger ones. The reverse process is apparent where this same product without ammonia was used. The ammonia-free Aglusol and SRS 88/52 were very similar in their effects and presumably are closely related chemically.

In the second experiment, where loess parental material was used, the salient features were the marked breaking down of the macro-aggregates during the three months course of the investigation and also the slight effect on this soil of the 0.01 per cent treatments, except in the case of SRS 88 52. These facts are demonstrated in Table III.

TABLE III

Soil Treatment	Percentage of water-stable aggregates in loess parental material					
	Commencement of Experiment			Conclusion of Experiment		
	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm
None	0.4	0.4	0.8	0.5	0.0	0.5
0.01% Aglusol	0.5	0.4	0.9	0.9	0.2	1.1
0.05% ,,	13.8	36.1	49.9	9.9	6.1	16.0
0.10% ,,	6.8	74.3	81.1	9.8	23.7	33.5
0.01% Ammonia-free Aglusol	1.2	0.7	1.9	1.8	0.5	2.3
0.05% Ammonia-free Aglusol	15.5	37.8	53.3	6.6	17.0	23.6
0.10% Ammonia-free Aglusol	11.9	72.5	84.4	9.4	39.2	48.6
0.01% SRS 88/52	10.4	5.2	15.6	0.7	0.2	0.9
0.05% SRS 88/52	11.4	45.3	56.7	6.9	7.3	14.2
0.10% SRS 88/52	10.9	66.8	77.7	9.7	23.1	32.8
0.01% Krilium *)	0.6	0.5	1.1	0.9	0.3	1.2
0.05% ,,	16.5	8.4	24.9	4.7	2.8	7.5
0.10% ,,	10.4	26.9	37.3	11.8	7.1	18.9

*) 83% of active principle

In spite of the care taken to eliminate all variables except that of soil aggregation and its associated effects, the plants in neither

of these two experiments showed any very marked differences in growth which could be associated with treatment. In the case of both lots of soil material there was enough clay present to allow the soil to shrink away a little from the sides of the pots. When both experiments were concluded after three months and the plants were harvested by carefully washing away the soil to get the complete plants out, it was found always that in the control pots the roots were concentrated at or near the surface of the cylinder of soil, having obtained thus fairly adequate aeration and presumably sufficient nutrients from the well enriched soil materials. In the pots of well aggregated soil the lucerne roots were widely distributed through the soil mass.

In both experiments the plants grown in the soil materials aggregated with 0.1 per cent and 0.05 per cent of the products in general had obviously larger nodules which were clearly pink and healthy as compared with the smaller and paler pink to white nodules common on the plants grown in the untreated soil. The numbers of nodules present were too great for counting but there did not appear to be any difference in numbers per plant or per pot associated with treatments. It was not practicable to cut off and weigh the typically small nodules produced by *Rhizobium meliloti* on lucerne or to devise un unbiassed rating system for the measurement of these differences.

By a concerted effort all plants were washed out quickly over a two day period and immediately prepared and dried. Differences in amount of production were commonly apparent, as in the case of an Aglusol without ammonia series grown in loess parental material and illustrated in Plate I. The natural Aglusol gave striking differences in favour of treatment on this very nitrogen-deficient material but, of course, its effect had to be discounted in view of the ammonia supplied by this product, the aggregation effect apparently being confounded with a nitrogen response.

The clean, whole plants were all cut at the hypocotyl and the tops and roots produced by each pot were dried to constant weight at 103°C and weighed. Careful analysis of the data concerning weights of total plants, tops and roots failed to reveal any worthwhile significant differences in favour of treatment or otherwise, except the former in the suspect case of the Aglusol treatments of the loess parental material, illustrated in Plate II.

In the third experiment, using surface soil, the earth was more compacted and the untreated and 0.01 per cent treated soil lots quickly became much denser at the surface. Although in this case also there was some shrinkage of the soil away from the pots, to the apparent advantage of the plants in the untreated soil, the dense stand of plants resulted in the roots penetrating most of the soil mass. Even though in this experiment also soil moisture was never allowed to be a limiting factor in the case of the less aggregated soil lots, marked apparent differences in plant growth occurred.

The soil used in this experiment was the same as that used in the first experiment and the levels of macro-aggregation of the variously treated soil lots at its commencement were the same as those given in Table II. The situation with respect to content of water-stable macro-aggregates one month and two months after plant growth had commenced is shown in Table IV. It was indicated

TABLE IV

Soil Treatment	Percentage of water-stable aggregates in surface soil Experiment 3					
	After one month			After two months		
	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm	0.5–1.0 mm	1.0–3.0 mm	0.5–3.0 mm
None	3.1	2.6	5.7	4.4	4.2	8.6
0.05% Aglusol	9.4	44.7	54.1	9.3	49.2	58.5
0.10% „	11.4	59.2	70.6	5.8	78.3	84.1
0.05% Ammonia-free Aglusol	9.6	42.2	51.8	7.2	55.7	62.9
0.10% Ammonia-free Aglusol	11.3	69.9	81.2	5.2	82.7	87.9
0.05% SRS 88/52	12.0	52.4	64.4	8.6	53.7	62.3
0.10% SRS 88/52	10.5	62.5	73.0	6.6	80.7	87.3
0.05% Krilium *)	11.7	13.4	25.1	10.3	15.9	26.2
0.10% „	19.9	37.1	57.0	16.8	40.3	57.1

*) 83% of active principle

strongly that there had been a rapid break-down of the macro-aggregates in the untreated soil and then a slow rebuilding of them as the root mass developed and influenced the situation, presumably as the result of the activity of the rhizosphere microflora. The soil lots treated with the three Belgian products showed that there had been appreciable building of smaller aggregates into larger ones over this period. The Krilium-treated lots showed a maintenance

Plate I. Variation in growth of sets of 25 lucerne plants grown three months in loess parental material treated with ammonia-free "Aglusol".
Left to right: Control, 0.01%, 0.05% and 0.1% treatments.

Plate II. Very significant increase in growth of lots of 25 lucerne plants over three monthscaused by treatment of loess parental material with normal "Aglusol" solution
Left to right: Control, 0.01%, 0.05%, and 0.1% treatments

Plate III. Comparison of average sets of 50 two month old lucerne plants taken at random from dense stands grown in topsoil.

Left: From control soil and with a total of 250 nodules. *Right:* From soil treated with 0 05% normal "Aglusol" and with a total of 652 nodules.

Plare IV. Similar to Plate III but showing the average difference found between control *(left)* and 0 1% normal "Aglusol" treatment *(right)*.

of macro-aggregation with the 0.05 per cent level and a definite increase with the 0.1 per cent level.

In this experiment four replicates were harvested one month after the growth of the lucerne commenced and the other four replicates were harvested one month later still. The plants grown in each pot were washed out carefully so as to remove all soil without any possibly avoidable loss of root material; each replicate was treated as a unit. Here again the plants coming from well aggregated soils had apparently larger and more obviously pink nodules than those grown in the less aggregated soils. As the plants of each pot were harvested, fifty were taken at random and the nodules present on each one were counted; then these plants were cut at the hypocotyl and the tops and roots were dried separately at 103°C.

Statistical analysis of the data obtained revealed no significant effects of any soil conditioning treatment, after one month, on numbers of nodules, weights of tops or total dry weights.

The data obtained from the lucerne plants grown for two months showed a significant effect of treatments on the number of nodules; there was some evidence of interaction between treatments and levels, the Aglusol and ammonia-free Aglusol series showing the greatest effects with the increase in level of treatment from 0.05 per cent to 0.1 per cent. It was found that the total dry weights of plants and the dry weights of the tops showed a parallel trend although the interaction between materials and levels did not reach significance in this case. On the evidence available one could speak with confidence of an increased yield over the controls only for the level 0.1 per cent of the Aglusol and ammonia-free Aglusol treatments.

With the second four replicates, as in the case of the first four, counts were made of the number of lucerne plants which became established in each pot and the number present at time of harvest. Treatments had significantly affected plant survival at the two months stage, the smallest losses caused by damping-off (due to species of *Pythium*, etc.) being in the untreated soil lots. However, there was no evidence of a correlation between the weights of tops of the fifty plant samples and the number of plants per pot between replicates of the various treatments; it is unlikely therefore that the effect of the above-mentioned treatments on yield is a consequence of the effect on survival and intensity of competition.

In the case of the lucerne seedlings harvested after two months the numbers of nodules were significantly greater even where the soil had been treated with natural "Aglusol" and therefore had received additions of ammonia. Plates III and IV illustrate the marked differences observed between the fifty random plant samples. On the left, in the former, the group of plants from the untreated soil had a total of 250 nodules and on the right the larger plants grown in soil treated with 0.05 per cent "Aglusol" had a total of 652 nodules. Similarly Plate IV shows the range of difference noted between fifty plants from a control pot (left) and fifty plants from a 0.1 per cent "Aglusol" pot (right). When the ammonia was removed from the "Aglusol" the differences in nodule numbers was of the same order but differences in plant vigour were less marked.

4. DISCUSSION

In general these pot experiments emphasised the difficulties involved in trying to simulate field conditions in pots.

Clearly there were not enough replications but it is difficult to see how a greater number of pots per treatment could ever compensate for the fundamental difficulty of shrinkage of the soil away from the pot to affect very greatly the air exchange and water intake situations to the advantage of the plants growing in untreated and poorly aggregated soil lots.

Although an accurate check was not kept on the amount of water applied, the control pots very obviously required a great deal more water for the growth of their plants than did those which represented the higher treatment levels. In the field a uniform precipitation might well have imposed the limiting factor of soil moisture deficiency on the plants growing in the less aggregated soil; at least it is reasonable to expect that the stress would have been greater on such plants.

In the field also where preparation of the seedbed and sowing of the seed is done by modern machinery the soil certainly would have been more compacted than it was in the pots of the first two experiments; this presumably would have allowed the well aggregated soil lots to show much greater advantages with respect to aeration.

Furthermore, had the soil been less fertile than that used in these

investigations and had the plants been grown for a longer period, it is reasonable to expect that the plants located in the cracking, poorly aggregated lots would not have been able to concentrate their roots at or near the surfaces and still obtain the nutrients necessary for as vigorous a growth as was made in these experiments.

The work has provided some good guidance for field experimentation on the effect of soil aggregation on legume nodulation and growth.

5. SUMMARY

1. Three pot experiments were conducted under glasshouse conditions to see if increased aggregation brought about by treatment of loam and its loess parent material with synthetic soil conditioners had any measurable effect on the nodulation and seedling growth of lucerne.

2. The effects of these substances on the initial and subsequent aggregation of the soil materials used was measured in terms of water-stable aggregates of diameter greater than 0.5 mm.

3. With these soil materials statistically significant differences in seedling growth at three months were not found when there was a relatively wide spacing of the plants.

4. However, when the plants were grown closely in topsoil so that the soil mass was fairly well occupied by roots, significant differences were obtained in the dried weights of two month old seedlings. Plant weights were increased as the result of soil aggregation by two of the treatments.

5. With these latter plants grown for two months there was a significant favourable effect of treatments on the number of nodules, there being some evidence of interaction between treatments and levels.

6. ACKNOWLEDGEMENT

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7. REFERENCES

1) Hanotiaux, G. et Manil, G., Note sur les résines synthétiques utilisées pour l'amélioration de la structure du sol. Bull. Inst. Agron. et Sta. Recherches Gembloux XX, 66–71 (1952).

2) Hely, F. W. and Bonnier, Ch., Influence of soil-aggregating substances on the population of gum-producing bacteria in a loess soil. Antonie van Leeuwenhoek **19**, 211–224 (1953).

3) Martin, W. S., Soil structure. East African Agr. J. **9**, 189–195 (1944).

4) Yoder, R. A., A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. J. Am. Soc. Agron. **28**, 337–351 (1936).

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UPTAKE AND TRANSLOCATION OF GRISEOFULVIN BY WHEAT SEEDLINGS

by ANNE STOKES

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In a preliminary communication, Brian, Wright, Stubbs and Way [5]) showed that the antibiotic griseofulvin, if applied to the roots of plants growing in culture solution, induced a considerable degree of resistance to fungal attack. Diseases controlled included those caused by *Botrytis cinerea* Fr. on lettuce, *Alternaria solani* (Ell. & Mart.) Jones & Grout on tomatoes and *Erysiphe graminis* DC on cereals (Brian [3]), Stubbs [13])). They further showed that if cereal seedlings were grown in culture solutions containing griseofulvin, griseofulvin or some substance showing exactly similar effects in a highly specific bioassay appeared in guttation drops produced at the leaf-tips. These and other observations indicated that the protective effect of griseofulvin applied to the roots was most probably due to movement of that substance in the plant tissue; in other words, that it acted directly as a systemic fungicide and not secondarily by altering the host metabolism. A study of the factors affecting the uptake and translocation of griseofulvin is necessary if its systemic fungicidal activity is to be understood.

Little is known of the translocation in plants of absorbed organic substances. The available information has been summarised by Mitchell and Marth [10]) and Arisz [1]). In general it appears that such substances, if introduced through the root system, are transported upwards with the sap-stream in the xylem, or, if introduced through the foliage, leave the leaf through the phloem, following the same path as the substances produced by photosynthesis. One of the main difficulties in studies of trans-

location has been the difficulty in locating the material whose translocation was to be studied.

Griseofulvin is an ideal substance for such work as it can be detected in low concentration and with great specificity by a simple bioassay (Brian, Curtis and Hemming[4], Brian[2]).

The present paper describes a technique for studying the factors influencing upward translocation of griseofulvin in wheat seedlings, on a quantitative basis, with a discussion of results so far obtained.

METHODS

Culture technique

The culture vessels used were of simple construction. A 'Perspex' cylinder (1.5 in. diam., 3 in. high) covered at one end by a piece of cotton gauze held in position by a rubber band, was placed in a 400 ml tall beaker (6 in. high) with the gauze-covered end uppermost. Nutrient solution was added to the level of the gauze, about 200 ml being needed. The nutrient used was a modified Pfeffer solution. The 'standard strength' nutrient had the following composition: $Ca(NO_3)_2$, 2.0 g; KH_2PO_4, 0.5 g; KNO_3, 0.5 g; KCl, 0.25 g; $FeCl_3$, 0.005 g; distilled water, 6 l. This nutrient was prepared in concentrated form and diluted for use as required. The nutrient was adjusted to between pH 6 and 7 with $N/10$ KOH before use. To each litre of nutrient, 1 ml of minor element solution (Hoagland and Snyder[7]) was added before use. Wheat seeds (*var.* Eclipse) were surface-sterilized in 0.1% mercuric chloride under pressure for 6 min and soaked in sterile water overnight. Twenty seeds were then set out on the gauze in each culture vessel and allowed to germinate in a glasshouse. After 7 days, ungerminated seeds and unhealthy seedlings were discarded, leaving 12–15 seedlings in each vessel. The nutrient was then discarded and replaced with more concentrated nutrient ($\times$ 3 or $\times$ 5 standard strength). After an appropriate time the nutrient solution was replaced by one containing griseofulvin or other test substances and thereafter these solutions were renewed every 2–3 days to ensure a more or less constant concentration of griseofulvin in the vicinity of the roots. Each treatment was replicated 3–5 times, each replicate consisting of two vessels (*ca.* 30 plants).

Detection of translocation

Every evening after the day in which the griseofulvin or other test substances were applied the culture vessels were placed in a humidity chamber to encourage formation of guttation drops. These were collected next morning in pasteur pipettes and stored at 1°C in a refrigerator until required for assay, a period which varied from 6 hours to 3 days. Each experiment lasted 10–12 days.

Preparation of griseofulvin solutions

Griseofulvin is only slightly soluble in water and the preparation of solutions of known strength was correspondingly difficult. However, solutions of 20–40 μg/ml concentration could be prepared by boiling the appropriate quantity of griseofulvin under reflux in water for 1 h. No decrease in griseofulvin concentration could be detected by bioassay under such conditions. The solution so prepared was added to nutrient solutions before use to give the required final concentration of griseofulvin.

Bioassay of griseofulvin

The technique described by Brian, Curtis and Hemming[4]) involved making serial dilutions with equal quantities of a suspension of *B. allii* conidia in a nutrient solution and observing the germination of the conidia after incubation for 18 h at 25°C. By comparing the characteristic distortions of growth with those produced in griseofulvin solutions of known strength the griseofulvin concentration in a given solution could be estimated; the accuracy of a single determination is probably not more than $\pm$ 50% but the mean values presented in this paper should have a much greater accuracy. In the present study, since only very small samples of guttation fluid were obtained (*ca.* 0.25 ml from each replicate) all dilutions were done on a much smaller scale than in the original technique, using micropipettes and small dilution tubes. The guttation fluid from plants which did not receive griseofulvin treatment invariably showed no griseofulvin activity in the bioassay.

RESULTS

Phytotoxicity of griseofulvin

When wheat seedlings were grown in griseofulvin at 5 μg/ml or higher concentrations the roots were stunted and swollen just behind the root-tip; the shoot was also stunted. Young wheat seedlings were more affected than older ones. At 20 μg/ml griseofulvin caused stunting and tip-burn of plants of all ages up to 7 weeks. Below 5 μg/ml there was no phytotoxic effect. Phytotoxicity of griseofulvin has been reported previously by Wright[14]) and Brian et al[5]).

Preliminary standardisation of technique

Concentration of griseofulvin applied. The results of an experiment are presented in Table I. The following points are worthy of notice: (*a*) when griseofulvin was applied at 5 or 20 μg/ml, two days elapsed before griseofulvin could be

TABLE I

Mean concentration (μg/ml) of griseofulvin in guttation drops from plants grown in nutrient solutions of varying griseofulvin content

Initial age of plants	Conc. of griseofulvin (μg/ml) in nutrient solution	Days after beginning of treatment					
		1	2	3	4	5	6
7 days	1	0	0	0	0.4	0.6	0.4
	5	0	0.3	0.4	3.0	3.0	3.0
	20	0	0.2	0.8	12.0	18.0	38.0
35 days	1	0	0	0.6	0.2	0.6	1.5
	5	0	0.2	0.6	1.5	4.5	18.0
	20	0	0.8	4.5	12.0	18.0	50.0

detected in the guttation drops, but when applied at 1 μg/ml this interval was consistently 1–2 days longer: (*b*) the higher the concentration of griseofulvin applied, the higher was the concentration found in the guttation drops; (*c*) the concentration of griseofulvin in the guttation drops tended to increase during the six days of the experiment; (*d*) the concentration of griseofulvin in the guttation drops in some cases eventually exceeded that in the solution applied to the roots, which together with (*c*), suggested accumulation of griseofulvin in the plant.

Since a concentration of 5 μg ml griseofulvin was slightly phytotoxic, and since with 1 μg ml the amount of griseofulvin which could be detected in guttation drops was rather small, in all subsequent experiments griseofulvin was applied to the roots at a concentration of 2 μg/ml.

Age of plant. The date in Table I indicate rather greater translocation in plants 35 days old at the beginning of the experiment than with 7 day old plants. The effect was small, and in later experiments 10–14 day old plants were always used.

Concentration nutrient solution. Uptake and translocation of griseofulvin was similar in 'standard strength' nutrient and in nutrient $\times$ 3 or $\times$ 5 that strength. Since the plants appeared greener and more vigorous in the more concentrated nutrient, that was used in subsequent experiments.

pH of nutrient solutions. The nutrient used had little buffering capacity, and whatever the initial pH of the solution, in the range pH 4–8, there was rapid drift towards neutrality after contact with the wheat roots. In order to investigate the

effect of pH on uptake and translocation of griseofulvin, therefore, pH was kept roughly constant by daily renewal of the culture solution. Under these conditions daily variations in pH did not exceed 0.5 unit.

No effect on griseofulvin uptake and translocation was observed in the range pH 5–8. Below pH 5 uptake and translocation of griseofulvin was slightly depressed. In all subsequent experiments an initial pH between 6 and 7 was always used, since drift of pH was then at a minimum.

Interval between sample collections. Collection of guttation drops involved placing the plants in a humid atmosphere overnight; with daily sampling the plants were therefore in a near-saturated atmosphere for 12–16 h in every 24 h. Occasionally a day's samples were unavoidably omitted, so that plants remained under normal conditions of atmospheric humidity for a longer period than usual. It was thought possible that this might affect results, so an experiment was set up in which plants kept otherwise under similar conditions were sampled at intervals of 1, 2, 3 and 4 days. The results are shown in Table II. Comparing

TABLE II

Effect of sampling frequency on mean concentration of griseofulvin (μg/ml) in guttation drops from plants grown in nutrient solution containing 2 μg/ml griseofulvin

Sampling interval (days)	Days after beginning of treatment											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	0	0.1	0.3	0.2	0.3	0.5	0.7	0.7	0.8	0.8
2	—	0	—	0.2	—	0.2	—	0.6	—	0.6	—	0.8
3	—	—	0.2	—	—	0.2	—	—	0.7	—	—	1.0
4	—	—	—	0.2	—	—	—	1.1	—	—	—	2.7

first the results obtained with daily sampling and sampling at 4-day intervals, it will be seen that with the less frequent sampling higher concentrations of griseofulvin were found in the guttation drops. This confirms the suggestion, made previously, that griseofulvin accumulates in the leaf. Results for 2-day sampling differed little from those of daily sampling and results for 3-day sampling were intermediate between those for daily and 4-day sampling.

Effect of environmental temperature and humidity

It became obvious as the work proceeded that the rate of movement of griseofulvin in the plant varied with the time of year, for griseofulvin could be detected in the guttation fluid 3 days after application in the summer but only after 5–6 days in winter. The effect of temperature and humidity was therefore investigated. These factors could not be adequately controlled with the apparatus available but certain definite trends could nevertheless be identified.

For these experiments 'Perspex' incubator boxes were constructed, each 6 ft. × 2 ft. × 2 ft., with a movable lid. The boxes had no bottom but stood on a perforated wooden base, larger in area than the base of the box, under which was fitted an electric heater controlled by a thermostat in the box. These incubators were kept in a glasshouse. The temperature in the boxes showed a considerable variation but it was possible to maintain a temperature difference of 10°C between two boxes fairly consistently. The relative humidity in the boxes could also be roughly controlled; by distributing dishes of fused calcium chloride in the incubator box an R.H. of about 50% could be maintained, and by spraying the interior with water and distributing water soaked cotton-wool between the culture vessels an R.H. of about 90% could be maintained.

The results of three experiments are presented in Table III. The concentration of griseofulvin in the guttation drops was about five times greater at 25 C than at 10 C, at normal atmospheric humidity in both cases. The griseofulvin concentration in guttation drops was about four times greater at 56% relative humidity than at 91% relative humidity, with an air temperature of approximately 25°C in both cases. Similarly, by increasing air movement with an electric fan, griseofulvin concentration was more than doubled at 10°C. Scarcely any griseofulvin was found in guttation drops of plants kept at the same temperature in a water-soaked atmosphere.

These results indicate that the rate of movement of griseofulvin in the plant is increased by factors which increase the rate of transpiration.

Effect of respiratory enzyme inhibitors

Recent work has shown that the uptake of nutrient ions by plant roots is linked with respiration and, if respiration is prevented or reduced by anaerobiosis or by the presence of suitable enzyme inhibitors, uptake of nutrient ions is also reduced. It was thought

TABLE III

Effect of atmospheric conditions on mean concentration (μg/ml) of griseofulvin in guttation drops from plants growing in nutrient solutions containing 2 μg/ml griseofulvin

Environmental condition varied	Days after beginning of treatment									
	1	2	3	4	5	6	7	8	9	10
A. *Temperature* (normal R.H.)										
10°C	0	0	0	0.5	0.4	0.2	0.3	0.4	0.4	0.6
25°C	0	0	0	1.2	2.2	2.3	3.1	2.4	5.7	5.1
B. *Relative Humidity* (at 25°C)										
56%	0	0	0.4	1.1	—	6.2	6.2	—	—	—
91%	0	0.1	0.3	0.4	—	1.3	1.4	—	—	—
C. *Air movement* (at 10°C)										
Moving air . . (normal R.H.)	0	0	0	0.5	0.3	2.0	1.8	3.4	—	8.5
Still air. . . . (normal R.H.)	0	0	0	0	0	0.5	0.8	1.3	—	3.2
Still air . . . (100% R.H.)	0	0	0	0	0	0	0.1	0.2	—	0.2

of interest to determine whether the uptake of griseofulvin, a relatively large neutral molecule, was similarly linked with respiration. Enzyme inhibitors were added at various concentrations with griseofulvin to the nutrient solutions. The range of inhibitor concentration was chosen after preliminary tests to determine the minimum level having a phytotoxic effect. The results of representative experiments are given in Table IV.

Sodium azide markedly reduced the amount of griseofulvin appearing in guttation drops at $10^{-5}M$ without noticeably affecting the growth of the plants or guttation. At $10^{-4}M$ the plants were severely stunted and no guttation took place; extracts of such plants were made but no griseofulvin could be detected. Phenylurethane markedly reduced the griseofulvin in the guttation drops at $10^{-6}M$ and higher concentrations. At $10^{-5}M$ and $10^{-4}M$ the plants were stunted but guttation was normal. Sodium fluoride did not significantly inhibit griseofulvin uptake and translocation in the range $10^{-6}M$ to $10^{-2}M$ and the plants were normal in appearance. 2,4-dinitrophenol at $10^{-5}M$ markedly reduced the

TABLE IV

Effect of respiratory enzyme inhibitors on mean concentration (μg/ml) of griseofulvin in guttation drops from plants growing in nutrient solutions containing the inhibitor and 2 μg/ml griseofulvin

Inhibitor and concentration	Days after beginning of treatment										
	1	2	3	4	5	6	7	8	9	10	11
Sodium azide											
None	0	0	0	0	0.3	0.2	0.2	0.4	0.8	0.9	—
$3 \times 10^{-6}M$	0	0	0	0	0.2	0.2	0.2	0.4	0.8	0.4	—
$10^{-5}M$	0	0	0	0	0.1	0	0	0.1	0.3	0.1	—
$10^{-4}M$	0	0	0	0	0	0	0	0	0	0	—
Phenylurethane											
None	0	0	0	0	0	0.4	0.5	0.8	—	1.1	1.0
$10^{-7}M$	0	0	0	0	0.1	0.3	0.3	0.4	—	1.4	1.6
$10^{-6}M$	0	0	0	0	0	0.2	0.1	0.4	—	0.7	0.7
$10^{-5}M$	0	0	0	0	0	0	0.1	0.1	—	0.7	0.7
$10^{-4}M$	0	0	0	0	0	0	0	0	—	0	0
Sodium fluoride											
None	0	0	0	0	0	0.1	0.2	0.5	0.5	0.3	0.4
$10^{-6}M$	0	0	0	0	0	0	0.2	0.4	0.4	0.3	0.3
$10^{-3}M$	0	0	0	0	0	0	0.2	0.6	0.5	0.4	0.3
$10^{-2}M$	0	0	0	0	0	0	0	0.5	0.4	0.4	0.5
2,4-dinitrophenol											
None	0	0	0.1	0.2	0.2	0.4	0.7	1.4	—	1.2	—
$10^{-7}M$	0	0	0.2	0.3	0.4	0.6	1.3	1.4	—	2.0	—
$10^{-5}M$	0	0	0	0	0.2	0.3	0.4	0.6	—	0.6	—
$10^{-4}M$	0	0	0	0	0	0	0	0	—	0	—

amount of griseofulvin in guttation drops. At $10^{-4}M$ the plants were severely stunted and no guttation took place.

DISCUSSION

The earlier work of Brian *et al.* [5] demonstrated that if oat seedlings were grown in a nutrient solution containing griseofulvin, after an interval of several days griseofulvin could be detected in guttation drops produced when the plants were placed in a humid atmosphere. The work on wheat seedlings now described increases our knowledge of this process in several respects, four of which are of special interest: (*a*) if the concentration of griseofulvin in the nutrient is increased in the range 1–20 μg/ml, the concentration of griseofulvin in the guttation drops also increases; (*b*) under

certain circumstances higher concentrations may be found in the guttation drops than in the nutrient solution; (*c*) factors increasing transpiration, such as increased temperature, decreased humidity, or air movement, increase the concentration of griseofulvin in guttation drops; (*d*) griseofulvin concentrations in the guttation drops are reduced if the roots are treated with respiratory enzyme poisons.

Though no study has been made of the relation between the concentration of griseofulvin in the guttation drops and its concentration in leaf tissues, it seems highly probable that the two are closely related. If that is the case, it follows that the various conditions which influence the concentration of griseofulvin in the guttation drops can also be considered to have a similar effect on the concentration of griseofulvin in the leaf tissues.

On that basis one can begin to form a picture of the sequence of events in the passage of griseofulvin from the nutrient solution to the leaves. The effect of respiratory enzyme poisons was generally similar to their effect on uptake of ions by excised or intact roots (Machlis[8]), Milthorpe and Robertson[9]), Nance[11]), Robertson, Wilkins and Weeks[12])) and on the uptake of water by potato tuber tissue (Hackett and Thimann[6])). Other workers have interpreted such results as meaning that uptake of ions or water is not a process of passive diffusion but that energy yielded by respiration is concerned in the process of uptake. It seems probable that the passage of griseofulvin molecules from the nutrient to the root is similarly linked with respiration. It is worth noting, however, that $10^{-4}M$ phenylurethane inhibited griseofulvin uptake without reducing guttation, which suggests that uptake of griseofulvin and uptake of water may not be directly linked.

None of the results presented here give any information about the mechanism by which griseofulvin passes from the superficial cells of the root through the root cortex, but the marked increase in griseofulvin content of the guttation drops under conditions favouring transpiration, indicates that the griseofulvin entering the root reaches the vascular system and passes upwards through the xylem to the leaves.

Finally, the fact that griseofulvin concentrations in the guttation drops tend to increase as the period of exposure of the roots to griseofulvin is prolonged, eventually on occasion reaching con-

centrations higher than that in the nutrient, is best explained by assuming that over such a period the root continues to take up griseofulvin, that this is translocated to the leaves and accumulates there.

SUMMARY

1. A roughly quantitative technique for studying uptake and translocation of the antibiotic griseofulvin by wheat plants has been devised. Wheat plants were grown in nutrient solutions containing griseofulvin and translocation measured by bioassay of the griseofulvin appearing in the guttation drops induced by transfer to a humid atmosphere.

2. Griseofulvin was phytotoxic at concentrations of 5 μg/ml and above, the first symptoms observed being stunting and swelling of the roots.

3. The concentration of griseofulvin in the guttation drops was directly related to the concentration in the nutrient solution; there was evidence of griseofulvin accumulation in the leaves, the concentration in the guttation drops being frequently higher than that in the nutrient solution.

4. Atmospheric conditions favouring transpiration increased uptake and translocation of griseofulvin.

5. Uptake and translocation of griseofulvin was inhibited by inclusion of respiratory enzyme inhibitors in the nutrient solution.

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REFERENCES

1) Arisz, W. H., Transport of organic compounds. Ann. Rev. Plant Physiol., **3**, 109–130 (1952).
2) Brian, P. W., Studies on the biological activity of griseofulvin. Ann. Botany N.S. **13**, 59–77 (1949).
3) Brian, P. W., Antibiotics as systemic fungicides and bactericides. Ann. Applied Biol. **39**, 434–438 (1952).
4) Brian, P. W., Curtis, P. J. and Hemming, H. G., A substance causing abnormal development of fungal hyphae produced by *Penicillium janczewskii* Zal. I. Biological assay, production and isolation of 'curling factor'. Trans. Brit. Mycol. Soc. **29**, 173–187 (1946).
5) Brian, P. W., Wright, J. M., Stubbs, J. and Way, A. M., Uptake of antibiotic metabolites of soil microorganisms by plants. Nature **167**, 347 (1951).
6) Hackett, D. P. and Thimann, K. V., The action of inhibitors on water uptake by potato tissue. Plant Physiol. **25**, 648–652 (1950).

7) Hoagland, D. R. and Snyder, W. C., Nutrition of strawberry plants under controlled conditions. Proc. Am. Soc. Hort. Sci. **30**, 288 (1933).
8) Machlis, L., The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation by excised barley roots. Am. J. Botany **31**, 183–192 (1944).
9) Milthorpe, J. and Robertson, R. N., Studies in the metabolism of plant cells. VI. Salt respiration and accumulation in barley roots. Australian J. Exp. Biol. Med. Sci. **26**, 189–197 (1948).
10) Mitchell, J. W. and Marth, P. C., Growth regulating substances in horticulture. Ann. Rev. Plant Physiol. **1**, 125–340 (1950).
11) Nance, J. F., Inhibiting nitrate assimilation in excised wheat roots by various respiratory poisons. Plant Physiol. **25**, 722–735 (1950).
12) Robertson, R. N., Wilkins, M. J. and Weeks, D. C., Studies in the metabolism of plant cells. IX. The effects of 2,4-dinitrophenol on salt accumulation and salt respiration. Australian J. Sci. Research B **4**, 248–264 (1951).
13) Stubbs, J., The evaluation of systemic fungicides by means of *Alternaria solani* on tomato. Ann. Applied Biol. **39**, 439–441 (1952).
14) Wright, J. M., Phytotoxic effects of some antibiotics. Ann. Botany N.S. **15**, 493–499 (1951).

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A SIMPLE METHOD FOR THE COMPARATIVE ESTIMATION OF SOIL WATER

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INTRODUCTION

A great deal of ecological work is perforce done far from a well-equipped laboratory, so that only the simplest techniques can be adopted. The soil moisture status is often a major ecological factor, but most modern field techniques involve the use of cumbersome electrical apparatus and predetermined points of measurement. For this reason, the ecologist usually falls back on the simple determination of percentage moisture by weight, in spite of the inherent limitations of this concept.

Though there are doubtless many agricultural workers who would be glad of a simple field method, the problem is greatest for those working with uncultivated soils. Any disturbance of the soil profile by the burying of instruments such as absorbent blocks, tensiometer pots or electrical elements is bound to upset permanently the local movement of water in the soil. To take an extreme example, the use of such techniques in the subsoil (C horizon) of a podzol involves the local disruption of the B horizon and the permanent disturbance of the effect of that horizon on water reaching the subsoil at that point. Results obtained from such instruments would therefore be meaningless.

The only answer to this problem is to use some method which will give a spot measure of the moisture status at the time of opening the soil section, and to open a new section each time further values are required. The method used must be quick-acting and easily transportable. Under these restrictive conditions high accuracy is not to be expected and is probably in most cases unnecessary. The author has designed a simple instrument which

meets most of these requirements, and has used it to his satisfaction in many field applications. It was briefly mentioned in an earlier article [2]) and the interest it aroused seemed to justify a fuller account.

DESCRIPTION AND METHOD OF USE

The instrument consists merely of a solid, pencil-shaped length of porous clay. Those so far used have been made by hand from No. 1 size Berkefeld filter candles; with care about 20 instruments can be made from one filter candle. The material is kieselguhr, with a considerable range of pore-size and with a total pore-space of about 50%. Doubtless other materials will be found to be equally suitable.

Each porous pencil has a cylindrical stem 11 cm long and 8 mm in diameter. One end is tapered to a point, the length of the point being 15 mm, giving a surface area of about 2 sq.cm. Along the stem a millimetre scale is stencilled with Indian ink (Plate I).

Whilst quite satisfactory results can be obtained in moist soils without treating the pencils in any way, in dryish soils it is sometimes difficult to detect the colour difference between the wet and dry material. Various methods of showing the rise of water up the stem by means of coloured dyes have been tried, but the only really satisfactory one is as follows. The clean dry pencils are dipped in an alcoholic solution of Cresol Red which has been slightly acidified. They are removed, dried in an oven at 100°C, and emerge a bright pink colour. This colour slowly fades in normal atmosphere, but even so, it lasts for several days. (In Plate I compare the tone of the pencil on the left, which had been exposed for several days, with that of the upper part of the right hand pencil, which was freshly coloured.) If, however, the pencils are sealed in an air-tight container with a little silica gel the colour is maintained indefinitely. When the pencils are in use, water rises up the stem by capillarity, the distance it has risen being marked by a clear-cut colour change from deep pink to pale yellow (Plate I). This is a simple pH-indicator colour change occurring at pH 0.2 - 1.8 and it is safe to assume that no soil solution will be encountered with a pH of less than 1.8.

The tapering point is inserted into the soil with as little dis-

Plate I. Porous pencils before and during use.

Plate II. Porous pencils in use in soil pit.

turbance as possible and so that the whole of the cylindrical stem projects. If the soil is moist enough, water will rise evenly along the stem. In practice it has been found best to leave the pencil in position for one hour, at the end of which time it is removed and the reading taken on the scale as indicated by the colour change. Once the instruments are inserted they can be left unattended, a considerable advantage in field work.

Since the forces extracting water from the soil and passing it up the stem of the pencil are essentially capillary, it is to be expected that the position of the pencil, whether vertical, horizontal or oblique, will not affect the rate of uptake. This is easily shown to be true by simple experiment, provided that the point is firmly gripped by the soil in the oblique or horizontal position. This factor will be discussed again below.

LABORATORY TESTS

The first criterion determining the successful use of this method is that the readings shall be reproducible. This has been fully tested in the laboratory and in the field, and it is safe to say that the errors between instruments are small compared with differences *(a)* in the mean readings in soils of differing water contents and *(b)* due to the heterogeneity of natural soils. The composition of the Berkefeld filters has occasionally been found to vary and it is therefore advisable in any one project to use pencils from the same batch. Such differences are detectable from the colour and texture of the material and are easily confirmed by doing a trial run in water. Table I shows the behaviour of six pencils working simultaneously in a large volume of water.

Differences between individual pencils tend to become rather more marked in soils approaching the limits of dryness at which they will work, but this is probably due in large part to the difficulties of mixing such soil homogeneously.

To demonstrate the behaviour of the pencils under different moisture tensions, several types of soil and sand were saturated and allowed to air-dry in a revolving drum. At intervals the soil or sand was transferred to open containers, packed as constantly and uniformly as possible, and at the same time a sample was weighed and oven-dried at 105 C to constant weight to give a

TABLE I

Readings on six porous pencils working simultaneously in water							
Time in min (T)	Reading in mm (D)						
	1	2	3	4	5	6	Av.
1	9	9	10	10	10	10	9.7
5	21	21	23	22	22	22	21.8
10	30	30	32	30	32	30	30.7
20	43	43	44	42	44	42	43.0
30	52	52	54	52	54	51	52.5
40	60	60	62	60	62	59	60.5
50	66	66	68	66	69	65	66.7
60	73	73	74	72	75	71	73.0

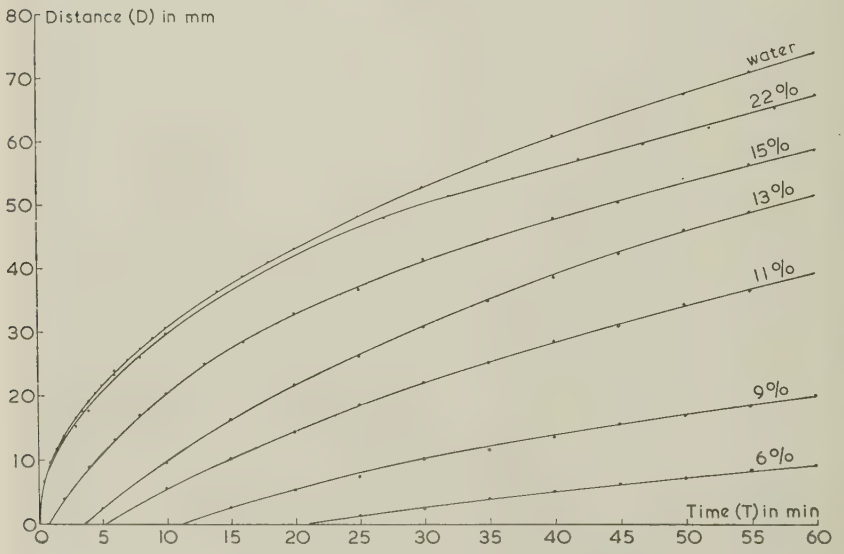

Fig. 1. Rates of water-uptake in water and in sand with different moisture percentages (by weight).

measure of the percentage of water by weight. Three pencils were inserted vertically and well-spaced in each container, and readings taken on them at frequent intervals. Fig. 1 shows the graphs obtained from the mean readings at successively drier stages in a sand with the curve for water to serve as comparison.

One of the greatest difficulties of laboratory testing in this way has been to achieve uniform and homogeneous packing of the soil. In fact this has not been really satisfactorily achieved,

and the laboratory figures must be regarded as approximate. A crude assessment of the importance of this factor was made in the laboratory on two materials — a fine garden loam and a sand. They were brought to such a state of dryness that when loosely packed the pencils just gave a reading in one hour. Each material was packed layer by layer in four containers under four different degrees of pressure; at the end of each test the weight and volume of soil was determined, so that a density calculation could be made. One pencil was inserted in each container and the one-hour readings taken. The results are expressed in Fig. 2. Both materials are obviously drastically affected by the degree of packing, but the loam, as one might expect, to a much greater extent than the

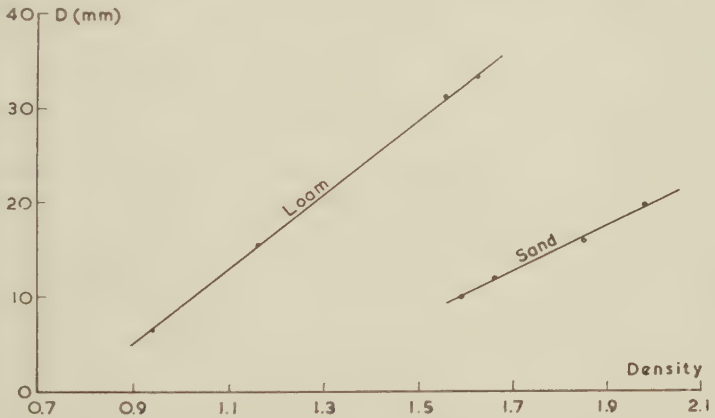

Fig. 2. Relationship between soil packing and pencil readings in loam and sand.

sand. The linear relationship between the pencil readings and density is remarkable, but since the readings are taken at an arbitrary time, the relationship is not regarded as of fundamental importance.

THEORETICAL CONSIDERATIONS

It will be apparent that these porous pencils work on essentially the same principle as the Livingston soil points [3]). The main difference is that the Livingston method involved weighing the points before and after use to give a gravimetric

estimate of the amount of water taken up in a given time. For field purposes the present method has obvious advantages; since it requires nothing more than a direct scale reading, it dispenses with weighing facilities and the necessity for strict precautions to avoid water loss from the instruments during transit. Since the mode of operation of the two methods is fundamentally the same, the extensive literature compiled by Livingston and his co-workers will be found to contain much that is relevant to the porous pencils also.

The curves shown in Fig. 1 are similar to those obtained by Bell and Cameron[1]) for the rise of liquids in absorbent materials such as blotting paper and soil columns. They found, as has been established with these pencils, that the rate of liquid movement is independent of gravity. Their curves were of the general form:

$$D^n = kT$$

(where D is the distance moved by the liquid interface in time T, n and k being constants).

By plotting log D against log T a straight line resulted from the curves of Bell and Cameron. But if this is done for the curves shown in Fig. 1 it is seen that the curves so obtained are not simple (Fig. 3). That for pure water is almost straight but is in fact composed of two straight lines intersecting at about 178°. A similar condition appears for the saturated soil, though the angle of intersection is smaller. In the other cases, however, the initial section is curved but eventually leads into a straight section which then continues indefinitely. In very dry soils this straight length may not be attained within the hour, but if readings are continued for longer it is found that eventually the rate of rise becomes approximately logarithmic in these soils too.

It seems likely that this difference in the form of the curves for liquid and for soil is connected with the establishment of continuity of capillary water between the soil pores and those of the pencils. However, no further analysis of these curves has been attempted here because

(*a*) the form of the curves probably depends on the soil packing, and, as already pointed out, this factor can only be regarded as approximately constant;

(*b*) no allowance is made for the wetting of the point of the pencil. It is apparent in Fig. 1 that the true origin of the curves is below zero.

Some consideration must now be given to the interpretation of results obtained by this technique. In the first place it must be made clear that no absolute calibration is possible in terms of any of the basic units by which soil moisture is usually measured. This

is because the behaviour of the pencils is governed by a complex of interacting factors; Livingston likewise encountered the same difficulty with his soil points. This may be a serious objection

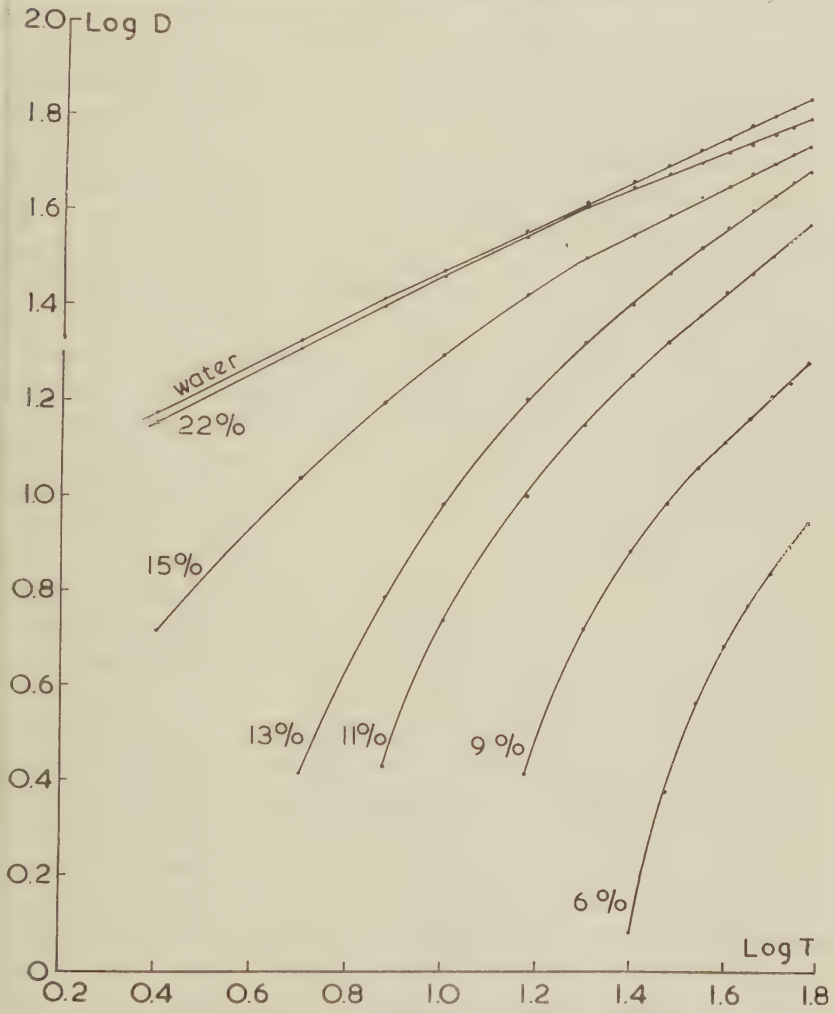

Fig. 3. Data of Fig. 1 expressed as logarithms of the variables.

to the instrument as far as the soil physicist is concerned, but it is in fact an advantage to the field worker who is seeking a measure of soil water in terms of its availability to the plant. No satisfactory physical measure has yet been devised which can give us a measure

of the ability of a soil to supply water over a long period of time; the most useful concept is the pF, but this measures the force retaining the water and not the water-supplying power of the soil. For example, though a soil mass may have a low pF, if that soil is clayey roots operating in it may in fact rapidly reach drought conditions due to the inability of the soil to make good local deficits rapidly enough by capillary flow. This factor does not enter into any of the techniques of pF measurement, but it will affect the behaviour of the porous pencils. In this sense the pencils are probably more akin to roots than other forms of instrument since they do what the roots do — remove water from the soil system over a period of time. On the other hand, the analogy must not be taken too far; earlier it was shown that dense packing of the soil increases the availability of water to the pencils, but by restriction of aeration, the effect on living roots might easily be to reduce the uptake of water. Nor can the porous pencil even approximate to the penetration of the soil by fine roots. Roots may exploit small pockets of moisture (*e.g.* in soil crumbs) which could not be differentiated by the pencils.

It is clear that no single method of measurement can give an estimate of the availability of water to plant roots under all field conditions, but it is felt that the comparative values attained with these pencils are a closer representation than is given by other more elaborate methods.

Owing to the difficulties of getting suitable standard materials it has not been possible yet to experiment with a range of porous clays. That used, however, fortunately covers most of the range of water-availability exploited by plants. It has been observed that wilting of grass occurs roughly at the point where the pencils fail to give a reading, so that zero values indicate conditions approaching wilting point. Certainly this material has proved useful under a wide range of field conditions, but more precise work in the region of the wilting point is called for. At the other end of the scale the method works well up to complete saturation of the soil.

USE IN THE FIELD

In the Introduction attention was drawn to the difficulty of obtaining reliable information about the water regime of natural

soils without upsetting the very factor one is trying to measure. This seems an insuperable difficulty at the moment, so it has been decided to accept the ideal as impossible of attainment, and deliberately to disturb the profile locally. But this is done not so that recording instruments can be inserted for future use, but so that spot measurements can be made of the water status at one time and one time only — the present. At the desired time, a soil pit is dug and the pencils inserted at once in the selected positions in a prepared face (Plate II). The readings will normally have been completed before the water regime is locally changed by the disturbance. For successive readings it is necessary to open a new pit each time as far as possible from any others, concomitant with the homogeneity of vegetation and soil. The pencils are used in duplicate or, preferably, triplicate, and may be inserted horizontally in a vertical face or vertically in steps cut at appropriate depths.

As indications of the type of measurements which can be made a few examples of field results will be quoted here. (These were made with pencils of an earlier pattern of a slightly different size from that detailed above, and the readings are not therefore directly comparable with those obtained in the laboratory tests described above.)

In the course of ecological studies of a certain area of moorland in Yorkshire it was found that there was a complex vegetation pattern which was a mosaic of two communities normally indicative of dry and wet habitats respectively. That these were in fact correlated with soil conditions was demonstrated by seasonal tests with the porous pencils. Table II gives a sample set of simultaneous readings. It is usual in these soils for the A_2 (bleached) horizon to give the highest values.

Another question which was quite simply answered in these

TABLE II

Comparison of soil moisture in wet and dry heathland communities

Horizon	Wet Site		Dry Site	
	Readings (mm)	Av.	Readings (mm)	Av.
A_0	57; 59	58.0	4; 0	2.0
A_2	70; 71	70.5	38; 34	36.0
B	49; —	49.0	24; 28	26.0
C	27; 22	24.5	11; 6	8.5

same soils was whether the iron pan acts as a complete barrier to the penetration of water to the subsoil. Sites as similar as possible were tested at different seasons, and the question is answered by the figures in Table III.

TABLE III

Seasonal readings beneath iron pan of heathland podzol						
Month (1949)	Jan.	Apr.	May	June	July	Nov.
Mean readings (mm) . .	42	22	30	8	25	70

There is clearly a seasonal swing which is in fact related to rainfall, indicating that the pan is not a complete barrier. On the other hand, comparison of surface figures with those in the subsoil would indicate that fluctuations are much less marked in the subsoil, so that the pan definitely obstructs the free flow of water downwards.

The method is especially applicable to localised variations in the soil which cannot be studied effectively by more cumbersome apparatus. For instance in these moorland soils old root channels are frequently met which are freely exploited by living roots. Table IV gives some measurements both in root channels and in the surrounding soil, and indicates the generally damper nature of these channels at any rate during the summer months.

TABLE IV

Comparison of moisture availability in old root channels and in surrounding soil					
Date	Horizon	Root channel		Surrounding soil	
		Readings (mm)	Av.	Readings (mm)	Av.
3/4/49	C	40; —	40.0	22; 21	21 5
10/6/49	C	29; 10	19.5	6; 8	7.0
14/6/49	C	24; 25	24.5	24; 21	22.5
20/7/49	B	80; —	80.0	49; —	49.0

Other uses to which the method has been successfully applied include comparisons of the seasonal variations in soil moisture under different types of forest cover on the same soil type and also between different soil types under the same cover.

SOURCES OF ERROR

It has already been made clear that errors due to differences between pencils are likely to be small compared with those arising

from soil heterogeneity, providing that the material from which the pencils are made is constant. Since the instrument only samples a small volume of soil it is liable to be more affected by variation in the soil than something larger. However, replication of instruments greatly reduces this drawback for general work, whilst there are many applications of these instruments for which small size is essential.

Unless regular cleaning is practised, it will be found that the pores of the point tend to clog. It has been found most satisfactory to soak the pencils in water (to remove the Cresol Red) followed by half an hour's boiling first in dilute hydrochloric acid and then in distilled water. If the condition does not yield to this treatment, as may be the case after much use in clay soils, a new point can easily be turned. This, of course, shortens the pencil by a few millimetres, but the full length of stem is rarely required.

Evaporation from the stem of the pencils appears to be negligible in normal circumstances. Working in soil pits offers some protection and on windy or sunny days the pits can be covered. For use under more exposed conditions a protective glass sheath can be fitted.

Perhaps the greatest source of error lies in the contact between the pencil and the soil. This must be good, and some soil materials are consequently unsuitable to study by this technique; loose raw humus under coniferous stands is one such instance. Some disturbance of the soil is inevitable on the insertion of the pencil, but this applies to all readings; best results are obtained by pushing the pencil into the soil with a twisting movement. It is usually possible to detect faulty contact when the pencil is being inserted, especially if the point has come up against a concealed stone or root. Livingston found it essential to use his soil points in a vertical position; otherwise contact was poor on one side. This does not appear to be necessary with the pencils, except possibly in poorly-binding material; being much less massive than the soil points they do not work loose when used horizontally in most soil materials. If an anomalous reading is obtained, close inspection of the point of insertion will usually reveal the source of trouble. Clearly errors of this type are liable to be most frequent in very stony soils, but even in these there are usually pockets of fine material which can be used.

SUMMARY

A simple instrument has been devised for the comparative estimation of soil moisture under field conditions where more elaborate techniques are not practicable. It consists of a solid length of porous clay, the size and shape of an ordinary pencil, pointed at one end and with a graduated scale along its length. The point is stuck into the soil, and the distance to which the water moves along the stem is used as a measure of the water-supplying power of the soil. Since it is not possible to calibrate in absolute units, the direct readings are used comparatively. Laboratory and field tests of the instrument are discussed.

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REFERENCES

1) Bell, J. M. and Cameron, F. L., The flow of liquids through capillary spaces. J. Phys. Chem. **10**, 658 (1906).
2) Dimbleby, G. W., Soil regeneration on the North-east Yorkshire Moors. J. Ecol. **40**, 331 (1952).
3) Livingston, B. E. and Koketsu, R., The water-supplying power of the soil as related to the wilting of plants. Soil Sci. **9**, 469 (1920).

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INVESTIGATIONS ON THE DENITRIFYING PROCESS IN SOIL

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INTRODUCTION

Until recently it has been difficult to determine quantitatively the extent of nitrogen fixation and denitrification in soil, because by conventional methods, only the sum of these two processes — their equilibrium state — is found. In order to observe these reactions independently, the heavy isotope of nitrogen, N^{15}, was used.

It is now generally agreed that denitrification implies only the production of nitrogen or gaseous oxides of nitrogen, through the reduction of nitrate, the nitrate acting as a hydrogen acceptor. This concept is the one which will be followed.

The literature of nitrogen transformations in synthetic media is extensive. Several schemes for denitrification have been proposed. Beijerinck and Minkman [5]) showed in 1910 that N_2O, as well as N_2, are liberated in cultures. They also showed that N_2O can be utilized by several microorganisms, when supplied with the appropriate substrate. They proposed the following scheme:

$$2KNO_3 + C.. \rightarrow 2KNO_2 + CO_2$$
$$2KNO_2 + C.. \rightarrow N_2O + K_2CO_3$$
$$2N_2O + C.. \rightarrow N_2 + CO_2$$

or the direct formation of N_2O from KNO_3:

$$2KNO_3 + 2C.. \rightarrow N_2O + K_2CO_3 + CO_2$$

Subsequent development of these basic concepts has to some extent altered their method of expression but has not changed the stoichiometry of the reaction. Although early work had shown the im-

portance of N_2O in the nitrogen cycle, surprisingly enough the possibility of large losses of nitrogen as nitrous oxide from soils is almost completely ignored in the soil literature. This may possibly be due to technical difficulties in the analysis of the small quantities of gas liberated from the soil and to the inert nature of N_2O itself.

Of the mechanism of soil denitrification even less is known, although some speculation is found in the literature. Newton Wood Barrit[4]) stated in 1931:

"The production of free nitrogen from organic compounds does not occur by bacterial action, but only indirectly by the interaction between free nitrous acid and amino compounds, both of which may be produced simultaneously by bacterial agency."

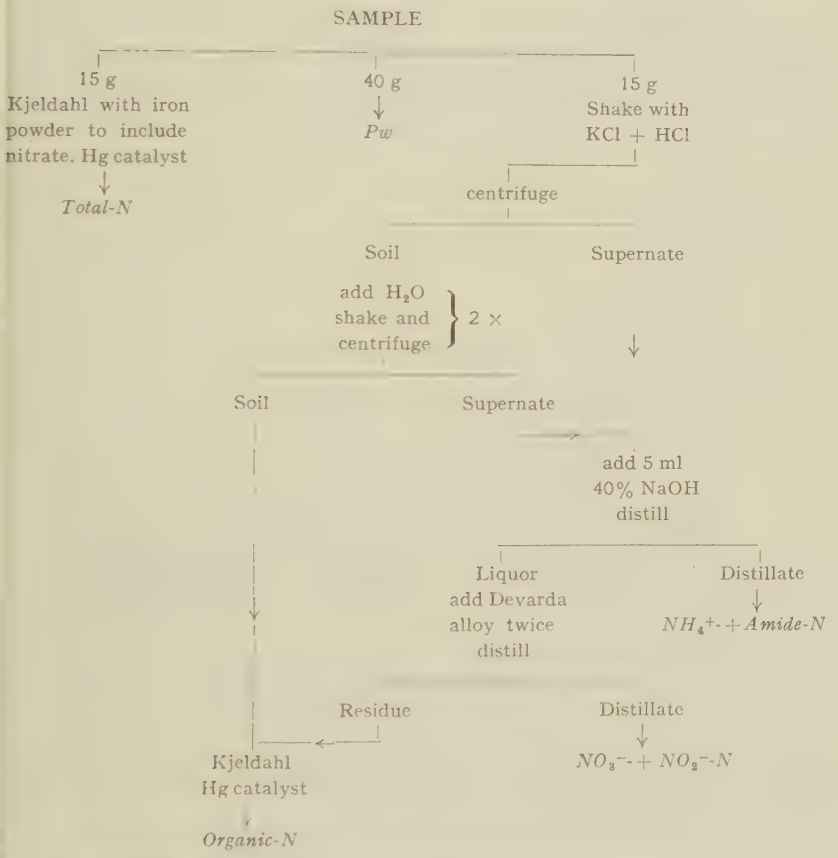
Both van Olden[12]) and Sacks[14]) and more recently Allen and van Niel[2]) have studied denitrification by manometric methods and have obtained quantitative data on pure cultures, the latter workers having developed probable intermediates and isolated cell-free systems carrying out some of the reactions.

Although most denitrification experiments were carried out anaerobically, Meiklejohn[11]), Broadbent[6])[7]) and Verhoeven[18]) stated that considerable denitrification could take place in aerated systems. Recent lysimeter investigations by Chapman, Liebig and Rayner[8]) gave indirect proof of denitrification. Reviewing the literature they reported unaccounted-for losses of nitrogen ranging between 25 and 100 pounds per acre per year. Their own experiments showed losses of about 60 pounds per acre per year.

METHODS

In order to simulate field conditions and still retain all products of denitrification, the apparatus shown in Figure 1 was designed. The soil and substrate are mixed and placed in bottle A. Adapter B, containing a KOH well, is used to fit the bottle to a closed arm manometer, C. Stopcock D, fitted on the manometer permits the admission of a gas atmosphere of any desired composition and also allows gas samples to be taken at any time. Changes in pressure in the bottle due to sampling, oxygen uptake, or denitrification can be followed on the manometer.

Methods of soil nitrogen analysis had to be selected, such that isotope determinations could be made on the fractions. The following scheme yielded sufficient quantities in the various fractions to permit mass spectrometric analysis.



Gas analyses and isotope distribution determinations were made with a Consolidated-Nier isotope ratio mass spectometer model 21–201.

For a typical experiment 200 grams of air dry unpasteurized soil were carefully mixed with the substrate chosen (dried and ground alfalfa or straw to provide adequate organic matter) and with a 10% inoculum of fresh garden soil. The microbial flora which developed was therefore determined by the substrate and other soil conditions, *e.g.* pO_2, pH, etc.

A fertile California soil, Yolo silty clay loam, was selected for these experiments to prevent, as much as possible, limitations due to mineral deficiencies. The pH of a 1 : 1 soil suspension is 7.2.

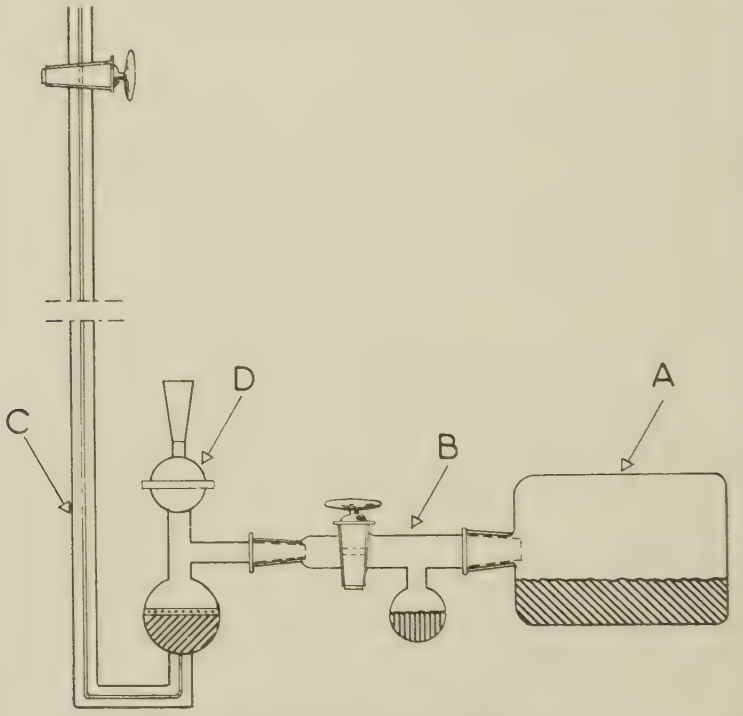


Fig. 1. Apparatus used to study soil denitrification.
Explanation in text.

The moisture equivalent is 24.6%. Nitrogen analysis of the soil gave the following results:

Fraction	Microequivalents nitrogen per gram dry soil
Nitrate ion.	3.21
Ammonium ion (incl. amides)	0.64
Organic nitrogen	59.1
Total nitrogen	63.0

All results have been expressed in microequivalents (μe) of nitrogen per gram dry soil. In terms of pounds per acre, 1 microequivalent nitrogen per gram dry soil equals approximately 28 pounds nitrogen

per acre (6 inches deep). Similarly, 1 milligram alfalfa or straw per gram soil is equivalent to 1 ton per acre, 6 inches deep.

RESULTS

Results of experiment 1 designed to compare two substrate levels are shown in Figure 2. When N^{15} in the atmosphere was determined at the end of the experiment, large isotope losses were found. Further mass spectrometric analysis of the atmosphere over the soil revealed

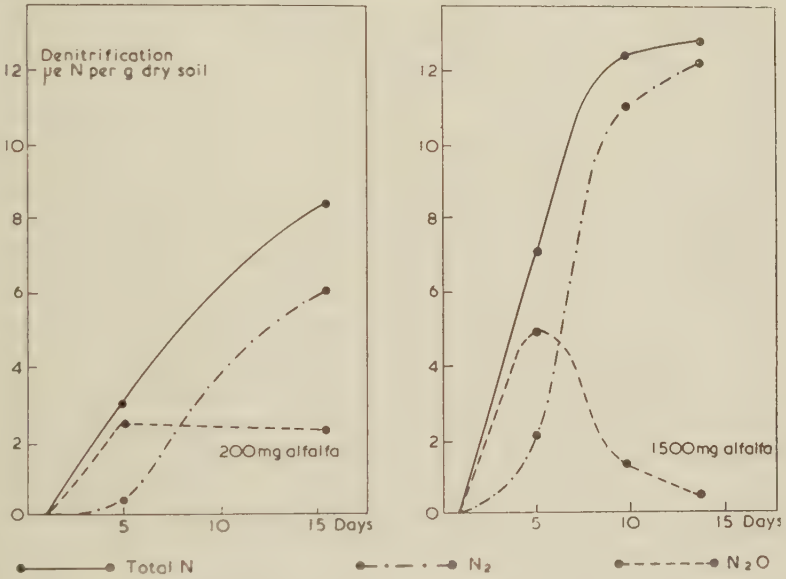


Fig. 2. Denitrification at two substrate levels (Exp. 1).
200 g Yolo soil; $P_w = 29\%$; pH = 7.2; NO_3 = 13.6 μe N per g dry soil.

the presence of considerable quantities of N_2O, which accounted for the observed loss and gave recoveries of 90% or higher. Although under field conditions much of the N_2O produced would have been lost to the atmosphere, the high alfalfa level experiment showed that N_2O could readily be further reduced in the soil.

Experiment 2 shows the effect of moisture content of the soil on denitrification. Four bottles were filled with Yolo soil and adjusted to progressively lower moisture levels. The bottles were left for 17 days, aerobically (pO_2 = 10-20 cm) at room temperature. Because

TABLE I

Experiment 2. The effect of moisture content on denitrification

At start of experiment all bottles contained: 200 g Yolo soil; 300 mg alfalfa; NO_3^- = 12.2 μe N/g dry soil; 20 cm N_2; pH 7.2. Bottles were left for 17 days under an oxygen tension of 10–20 cm. Subsequently they were left for 28 days under a nitrogen atmosphere

Denitrification under pO_2 of 10–20 cm				Denitrification in absence of oxygen				
P_w %	microequivalents nitrogen per gram dry soil			P_w %	microequivalents nitrogen per gram dry soil			
	N_2O	N_2	Total N		N_2O	NO	N_2	Total N
28.8	0.25	0.58	0.83	21.6	—	—	2.68	2.68
21.8	0.57	0.01	0.58	17.7	0.85	—	1.67	2.52
14.8	0.27	—	0.27	10.7	1.67	0.17	1.36	3.20
9.8	—	0.04	0.04	5.6	0.39	0.15	0.08	0.62

denitrification at this higher oxygen level was exceedingly slow, the gases were removed at the end of the experiment and nitrogen was substituted. The soils were then studied for 28 days under anaerobic conditions. The results are shown in Table I. The observed dependence of denitrification on moisture content in the presence of oxygen is most readily explained by the effect of moisture content on the rate of oxygen diffusion. This is demonstrated by the pronounced increase in denitrification with increasing moisture content in the aerobic soils as contrasted with the absence of any effect in the anaerobic soils. It is seen, however, that below the permanent wilting point limiting moisture directly suppressed denitrification.

In contrast with culture studies, where the production of N_2O is associated with high nitrate concentrations (1–10%), N_2O was produced in the soil at concentrations as low as 0.01% nitrate nitrogen in the soil solution.

A third denitrification product was identified as nitric oxide (NO). It was found at low moisture levels and only under anaerobic conditions.

The effect of soil pH on denitrification is not well known. It is generally assumed that denitrification is favored by a neutral to alkaline reaction. Jansson and Clark[9] did not observe denitrification in the decomposition of alfalfa or straw under acid conditions, even at moisture saturation. No effect on the distribution

of the liberated compounds has been reported. Non-enzymatic denitrification:

$$R\text{–}NH_2 + HNO_2 \rightarrow ROH + N_2 + H_2O$$

(van Slyke reaction)

and

$$NH_2OH + HNO_2 \rightarrow N_2O + H_2O$$

is favored by a low pH. However, Allison and coworkers [3]) found the chemical production of nitrogen to be negligible within the range of acidities of normal soils.

Experiment 3 demonstrates the influence of pH on denitrification. Yolo soil was adjusted to several pH levels by shaking overnight with H_2SO_4 or KOH and subsequent drying. Five bottles were set up with this soil adjusted to different pH levels. The same amount of substrate, nitrate and moisture was added to each soil, after which they were left for two weeks under anaerobic conditions at room temperature. Figure 3 shows the course of denitrification for several pH ranges. (During denitrification the pH of the soil increases, therefore both original and final pH values are indicated.)

It is shown that above pH 6 total denitrification rates were quite constant. Under the conditions of the experiment almost all nitrate was lost in less than two weeks. Below pH 6 denitrification rates were retarded slightly, but even at pH 4.9 more than 70% of the nitrate was lost within two weeks.

The effect of pH on the distribution of the various gases that were formed was very pronounced. Below pH 7, N_2O was the major product of denitrification. Above the neutral point N_2O was still produced in excess of N_2 but it was subsequently further reduced to N_2. This latter reaction is apparently inhibited by acid conditions. It should be kept in mind that under normal field conditions anaerobiosis increases with depth and nitrous oxide might be more easily reduced during its upward diffusion through neutral or basic soil.

An interesting point is the production of NO at low pH. Almost equal volumes of NO and N_2O were liberated at pH 4.9. (Fig. 3 shows gas production in microequivalents nitrogen per gram dry soil.) The formation of NO at this low pH may be a chemical decomposition of microbiologically produced nitrite:

$$3HNO_2 \rightleftharpoons 2NO + HNO_3 + H_2O$$

However, at low moisture levels NO was also produced at pH 7.2 (see Table I), which would indicate that it was probably formed enzymatically. With respect to the production of NO it may be

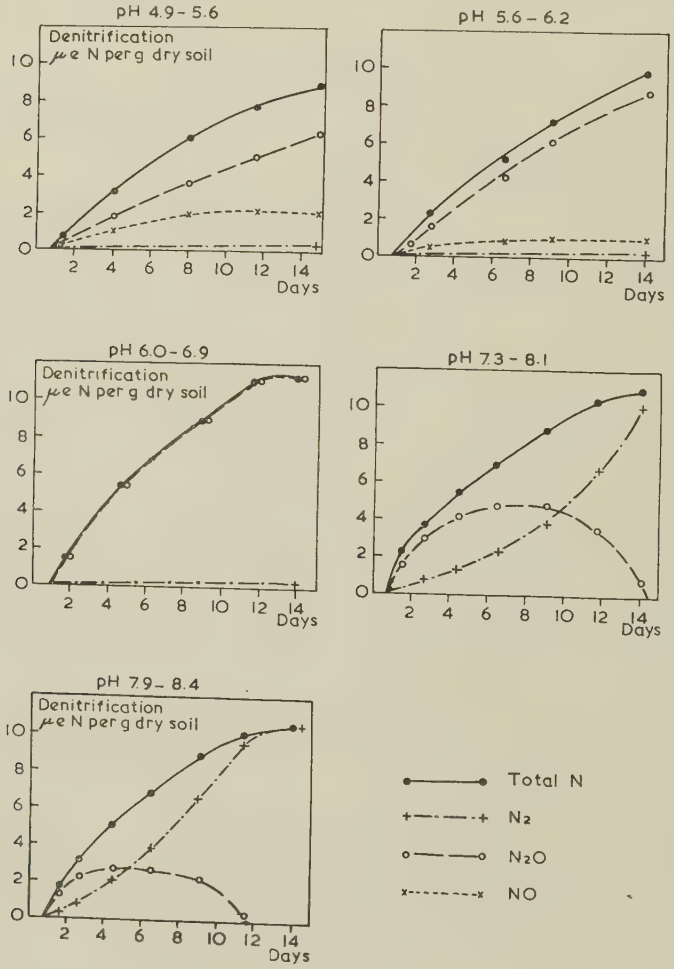


Fig. 3. The effect of soil pH on denitrification (Exp. 3). 130 g Yolo silty clay loam + 200 mg alfalfa; P_W = 17%; NO_3^- = 13.0 μe N per g dry soil.

noted that an old reference by Tache[16]) on the production of nitric oxide during the fermentation of beets was not corroborated by the work of Beijerinck and Minkman[5]) and Su-

zuki [15]), a fact which resulted in some correspondence involving these workers. Recently Verhoeven [18]) reported the occurrence of reddish brown fumes in the fermentation of beet molasses. The data of experiments 2 and 3 show that at least under some conditions the production of NO does indeed take place. In some cases considerable amounts of this compound may be formed. No critical delineation of the conditions under which it is produced is possible at this time, but its formation is also confirmed by the work of Allen (verbal communication). Subsequent experiments have shown that NO is further reduced to N_2, but this appears to be a slow reaction. Under field conditions, NO will probably diffuse and react with O_2 to give a variety of products.

The effect of oxygen on denitrification has been the object of numerous studies. Meiklejohn [11]) and Verhoeven [18]) reported considerable denitrification working with aerated liquid cultures. Broadbent [6]), working with aerated soil, found that below 4.5% oxygen all nitrate disappeared from the soil. Broadbent and Stojanovic [7]) recently studied the effect of oxygen tension on two New York soils and found denitrification of added nitrate to be inversely related to the partial pressure of oxygen. They also reported appreciable nitrogen losses at high oxygen levels. Sacks [14]), using liquid cultures of *Pseudomonas denitrificans*, found that an atmosphere containing 4% oxygen suppressed denitrification rates 50%. Other agricultural scientists [19]) have assumed denitrification to be negligible in well aerated soils.

To investigate this problem, six identically prepared bottles of soil were studied under different oxygen tensions (experiment 4). Some variations in the oxygen tensions could not be avoided and may account for slight divergencies in the results which are shown in Table II. In contrast with the results obtained with liquid cultures, or Broadbent's experiments, even an oxygen tension of 0.5 cm Hg inhibited the rate of denitrification to almost one-tenth of the anaerobic denitrification rate. At 2.0 cm O_2 denitrification rates were further reduced to about one-twentieth of the anaerobic rate of denitrification. It seems probable from Table II that denitrification is not altogether suppressed even under atmospheric conditions. The occurrence of small anaerobic volumes within the soil may well account for this residual denitrification.

The moisture level of this soil was between the moisture equivalent and the permanent wilting point. Had the moisture content been

TABLE II

Experiment 4. The effect of oxygen tension on rate of denitrification			
At start of experiment all bottles contained: 100 g Yolo soil; 200 mg alfalfa; NO_3^- = 12.2 μe N per g dry soil; 20 cm N_2; P_w = 20%; pH 7.2			
pO_2 (cm Hg)	Sum of N_2O and N_2 formed microequivalents nitrogen per gram dry soil		
	2 days	4½ days	14 days
0 (Anaerobic)	3.89	9.34	9.15
0.5	0.46	1.04	3.06
1.0	0.30	0.90	1.76
2.0	—	0.56	0.84
3.0	—	—	0.14
5–10	—	—	0.12

higher, higher figures would have been found for denitrification (as shown in Table I) because the diffusion of oxygen into the soil would have been impaired. The fact that denitrification in soil is far more inhibited by oxygen than it is in liquid cultures is most readily explained by the greater accessibility of oxygen in the porous medium. A secondary factor is the competitive utilization of the substrate by non-denitrifying aerobic organisms.

Table III shows the composition of the gases produced in ex-

TABLE III

Experiment 4. The mole percentage of nitrous oxide in the denitrification products			
At start of experiment all bottles contained: 100 g Yolo soil; 200 mg alfalfa; NO_3^- = 12.2 μe N per g dry soil; 20 cm N_2; P_w = 20%; pH 7.2			
pO_2 (cm Hg)	Mole % N_2O in denitrified gases		
	2 days	4½ days	14 days
0 (Anaerobic)	88	52	0
0.5	87	84	83
1.0	83	74	75
2.0	77	70	68
3.0	—	—	71
5–10	—	—	67

periment 4. It is seen that N_2O is the major product of denitrification comprising at least two-thirds of the gas liberated. In the anaerobic bottle 88% of the gas liberated in the first two days consisted of N_2O. If N_2O and N_2 had been allowed to escape, denitrification would have been complete in 4½ days. In this bottle, however, after the nitrate level had become somewhat depleted, the reabsorption of N_2O and its conversion to N_2 assumed more prominence until after two weeks all of the N_2O had been reduced to N_2. Under field conditions, it would be expected that large quantities of N_2O would be lost to the atmosphere. Since N_2O is quite soluble in water, submerged soils, if not too acid, may favor further reduction of N_2O to N_2. Whether N_2O or N_2 is the major product of denitrification will then depend upon the rate of exchange of soil gases with the atmosphere. Arnold (private communication) and Taylor and coworkers [17]) have made analyses of soil gases and both of these workers reported the presence of N_2O. Adel [1]) discussed the potential contribution of these sources to atmospheric N_2O. Repaske and Wilson [13]) discussed the possible significance of this gas insofar as the inhibition of nitrogen fixation by *Azotobacter* is concerned.

TABLE IV

Experiment 5. The effect of nitrate concentration on denitrification

At start of experiment each bottle contained: 100 g Yolo soil; 200 mg alfalfa; 20 cm N_2; $P_w = 20\%$; pH = 7.7. Microequivalents nitrate present per gram dry soil are indicated below

Nitrate μe N/g dry soil	Sum of N_2 and N_2O produced microequivalents N/g dry soil — Time in days							Mole % N_2O in denitrified gas above the soil — Time in days						
	0.5	1.0	1.5	3	6	12	23	0.5	1.0	1.5	3	6	12	23
19.3	—	—	3.44	6.45	8.99	12.5	13.7	—	—	94	95	88	66	28
10.2	—	—	4.60	7.48	8.00	8.00	—	—	—	87	63	5	0	—
5.6	0.20	1.74	3.46	4.05	—	—	—	?	86	53	0	—	—	—
3.4	0.40	1.55	2.46	—	—	—	—	53	21	0	—	—	—	—

Experiment 5 shows the effect of nitrate concentration on denitrification. It is seen in Table IV that the rate at which the gas is produced is not dependent on the initial nitrate concentration. The right hand section of the table shows the mole percentage of N_2O present in the gas above the soil. It is observed that N_2O is produced at all levels, although at lower concentrations the per-

centage N_2O was depressed and it was more rapidly further reduced.

No detailed study was made of organic substrates, but alfalfa was compared with straw as two representatives of typical mulching procedure. Table V shows that when straw is used for a substrate instead of alfalfa, the rate of denitrification is considerably reduced. The mole percentage N_2O produced is almost identical however for either substrate.

TABLE V

Comparison of alfalfa and straw as substrates for denitrification

At start of experiment each bottle contained: 100 g Yolo soil; 200 mg substrate; NO_3^- = 10.2 μe N/g dry soil; 20 cm N_2; P_w = 20%; pH 7.7

Substrate	Sum of N_2 and N_2O produced microequivalents N/g dry soil					Mole % N_2O in denitrified gas above the soil				
	1½ days	3 days	6 days	12 days	23 days	1½ days	3 days	6 days	12 days	23 days
Straw	2.11	3.46	4.54	5.66	7.11	85	83	76	53	20
Alfalfa	4.60	7.48	8.00	8.00	—	87	63	5	0	—

TABLE VI

Isotope content of denitrification products as compared with isotope content in nitrate fraction

Experiment	N^{15} content of nitrate	N^{15} content of denitrification products		
		N_2	N_2O	NO
3 — (pH 7.9 — 8.4)	21.2	20.2	21.0	—
(pH 5.6 — 6.2)	25.0	—	26.9	24.7
(pH 4.9 — 5.6)	26.9	—	28.1	28.1
4 — (anaerobic)	26.9	—	27.0	—
(pO_2 = 0.5 cm)	25.4	—	25.4	—
6 — (H_2 atmosphere)	26.9	26.6	26.8	—

It is of interest to know from which source the nitrogen, contained in the denitrification products, was derived. An analysis of the N^{15} content of N_2O, NO and N_2 produced in the foregoing experiments (see Table VI) showed that their isotope content always closely paralleled the isotope content of the nitrate. In experiment 6 (Table VI) 20 cm H_2 was substituted for 20 cm N_2 usually admitted, so that the exact isotope content of the liberated nitrogen gas could be determined directly. It can therefore be concluded that in these experiments both nitrogen atoms in N_2O and N_2, as well as the nitrogen atom in the NO were exclusively derived from nitrate.

In the presence of oxygen the oxidation of NH_3 to nitrate is very rapid. As a result of this, alternating aerobic and anaerobic soil conditions or low oxygen tensions may result in greater total nitrogen losses than would be found under strict anaerobiosis. It was indeed found that at low oxygen tensions, the isotope content of the nitrate became diluted, and the denitrified gases would show identical isotopic dilutions. Under anaerobic conditions some ammonia always accumulated in the soil. This was shown, by its very low isotope content, to be a decomposition product of alfalfa. Apparently no interaction between NH_3 and HNO_2 to produce N_2 took place even at a low pH, since no dilution of the gas produced was observed. These results agree with Jones [10] who also found N_2 to be derived from nitrate.

Only minor amounts of nitrate were reduced to ammonia, even when all nitrate disappeared. Nitrate reduction to ammonia was only observed to any extent at extremely high substrate levels (1500–6000 mg alfalfa per 200 g soil) under anaerobic conditions. Incorporation of labeled nitrogen into the organic fraction was always small.

SUMMARY

1. Following denitrification quantitatively with $KN^{15}O_3$ as a tracer, three products were identified: nitrous oxide, nitrogen and nitric oxide. The first can be further reduced to nitrogen under suitable conditions. Although apparently NO also is slowly further reduced, additional study is necessary to elucidate the significance of such reduction.

2. Nitrous oxide was found to be the major product of denitrification under most soil conditions. It was produced from soil containing as little as 3.4 microequivalent nitrogen per gram dry soil.

3. The moisture content of the soil indirectly affected denitrification by inhibiting the diffusion of oxygen into the soil, resulting in more denitrification. Below the permanent wilting point limiting moisture depressed denitrification directly.

4. Total denitrification rates were quite constant above pH 6, but the proportion of N_2O and N_2 were pH dependent. Above the neutral point nitrous oxide could be readily reduced to nitrogen. Below pH 7, the reduction of N_2O was strongly inhibited. Below pH 6 the formation of nitric oxide became more prominent and the rate of denitrification was decreased.

5. The suppressing effect of oxygen on soil denitrification was found to be very pronounced. Oxygen tensions as low as 5 mm Hg decreased denitrification to rates almost one-tenth of the anaerobic denitrification rate.

6 Under the conditions of these experiments initial nitrate concentration did not influence the rate of denitrification.

7. Although the use of straw instead of alfalfa as a substrate reduced the rate of the denitrification, the proportions of N_2 and N_2O formed were essentially unchanged.

8. Isotope content of all denitrification products showed them to be wholly derived from nitrate, indicating that a non-enzymatic denitrification (van Slyke reaction) is not taking place.

9. Reduction of nitrate to ammonia was found to be very small, even when all nitrate was lost during denitrification. Nitrate was reduced to ammonia in larger amounts only when excessive amounts of organic matter were added under anaerobic conditions. Incorporation of labeled nitrogen into the organic fraction was always very small.

10. Rapid oxidation of ammonia to nitrate may result in higher nitrogen losses under conditions of fluctuating oxygen tensions than under strict anaerobiosis.

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REFERENCES

1) Adel, A., A Possible Source of Atmospheric N_2O, Science **103**, 280 (1946).
2) Allen, M. B. and van Niel, C. B., Experiments on Bacterial Denitrification, J. Bact. **64**, 397–412 (1952).
3) Allison, F. E., Doetsch, J. H. and Sterling, L. D., Nitrogen Formation by Interaction of Nitrites and Amino Acids, Soil Sci. **74**, 311–314 (1952).
4) Barrit, N. W. The Liberation of Elementary Nitrogen by Bacteria, Biochem. J. **25**, 1965–1972 (1931).
5) Beijerinck, M. W., and Minkman, D. C. J., Bildung und Verbrauch von Stickoxydul durch Bakterien, Centr. Bakt. Abt. II, **25**, 30–63 (1909–10).
6) Broadbent, F. E., Denitrification in Some California Soils, Soil Sci. **72**, 129–137 (1951).
7) Broadbent, F. E. and Stojanovic, B. F., The Effect of Partial Pressure of Oxygen on Some Soil Nitrogen Transformations, Proc. Soil Sci. Soc. Am. **16**, 359–363 (1952).
8) Chapman, H. D., Liebig, G. F. and Rayner, D. S., A Lysimeter Investigation of Nitrogen Gains and Losses Under Various Systems of Covercropping and Fertilization, and a Discussion of Error Sources, Hilgardia **19**, No. 3 (1949).
9) Jansson, S. L. and Clark, F. E., Losses of Nitrogen During Decomposition of Plant Material in the Presence of Inorganic Nitrogen, Proc. Soil Sci. Soc. Am., **16**, 330–334 (1952).
10) Jones, E. J., Loss of Elemental Nitrogen from Soils Under Anaerobic Conditions, Soil Sci. **71**, 193–196 (1951).

11) Meiklejohn, J., Anaerobic Denitrification, Ann. Applied Biol. **XXVII**, 558–573 (1940).
12) van Olden, E., Manometric Investigation on Bacterial Denitrification, Proc. Koninkl. Akad. Wetenschappen Amsterdam, **XLIII**, 635–644 (1940).
13) Repaske, R. and Wilson, P. W., Nitrous Oxide Inhibition of Nitrogen Fixation by *Azotobacter*, J. Am. Chem. Soc., **74**, 3101–3103 (1952).
14) Sacks, L. E., Metabolic Studies on Denitrification, Thesis, University of California (1948).
15) Suzuki, S., Uber die Entstehung der Stickoxyd im Denitrifikations-Process, I, Centr. Bakt. Abt. II, **31**, 27–49 (1912).
16) Tache, B., Bildung und Verbrauch von Stickoxydul durch Bakterien, Centr. Bakt. Abt. II, **26**, 236 (1910).
17) Taylor, R.C., Brown, R.A., Young, W.S. and Headington, C.E., The Mass Spectrometer in Organic Chemical Analysis, Anal. Chem. **20**, 396–401 (1948).
18) Verhoeven, W., Aerobic Sporeforming Nitrate Reducing Bacteria, Thesis, Technische Hogeschool, Delft, Holland (1952).
19) Waksman, S. A., Soil Microbiology, p. 189, Wiley & Sons, New York (1952).

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ADAPTATION OF SWEET CORN TO ENGLISH CLIMATIC, EDAPHIC AND BIOTIC FACTORS

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HISTORICAL

Sweet corn, which is maize (*Zea mays*) with sugary seeds, has attracted interest in England not more than twenty years since initially grown at Merton, Surrey, by F. G. Brieger with encouragement from Sir Daniel Hall. Then C. D. R. Dawson commenced larger scale investigations, assorting out more adaptable North American varieties and testing their suitability for making hybrids, whose popularity was then spreading. Dawson produced two top-cross hybrids, made by pollinating varieties Extra Early Bantam or Canada Gold with Inbred C 13, bred by W. R. Singleton in Connecticut. These were released commercially in 1945 as John Innes Hybrid 1 and 2 respectively, the more popular Canada Gold hybrid also being called Canada Cross (Dawson[5]).

K. Mather and the writer then started a series of investigations, which included a programme of selection for cold tolerance. They found this effective only if desirable quantitative genes were already in the initial stocks. These were detectable by sowing under conditions previously unknown to the crop (Mather and Haskell[17]). Hence problems of disease and pest resistance of sweet corn and other types of maize in England were seen to be primarily those of adaptation.

PROBLEMS OF ADAPTATION

Adaptation and resistance to diseases and pests in a new environment depend on physiological reactions to new conditions

of temperature, day length and light intensity. Ultimately these rest upon the genetic basis by which selection acts. The breeder is therefore limited in what improvement he can achieve, unless genetical segregations or mutations occur readily in an introduced crop. In this respect there is greater chance of success in adaptating maize, an out-breeding species carrying heterozygous recessive genes, than tomato, an inbreeder whose limited ancestry affords little scope for selection.

Those open-pollinated, hybrid and inbred varieties of sweet corn most likely to suit English conditions come from experiment stations in a region embracing New England and Southern Ontario; this area extends from latitude 42° to 45°, or about seven to ten degrees less than for England (Table I). Sweet corns of flint an-

TABLE I

Earliness of Sweet Corn Varieties in England classified according to North American Origin

(data from Haskell[7])

Source	Number of varieties	Mean Earliness	Range
Experiment Stations in:			
Ottawa, Canada	6	12.8	5–25
Connecticut	11	13.5	0–28
Wisconsin	5	41.2	25–51
Seedsmen in:			
N. Dakota & Minnesota	5	21.0	7–28
Oregon	3	21.3	11–39
Up-State New York	13	22.8	7–51
Iowa, Illinois & Michigan	16	25.1	11–51
Connecticut & Massachusetts	7	27.7	7–50
Pennsylvania	5	29.0	22–51

Earliness, measured by days after 30 August 1948, was judged as time when most ears were ready for eating.

cestry are more suitable, as those from dent maize are often too late and may lack initial cold tolerance (unpublished data). In some ways sweet corn is better off in England than in America; thus several diseases such as leaf blight have not been reported, and smut is rarer in spite of the wetter climate. This may be because only small, comparatively isolated crops grow here, preventing possible epidemics. There is also the paradox that in the U.S.A. maize is attacked by corn earworm and corn borer, but not by frit

TABLE II

Life-cycle of Sweet Corn in England in relation to Disease and Insect Attacks

Stages in Life-Cycle	Physiological Setbacks	Attacks due to			
		Diseases	Insects	Rodents	Birds
Germination and emergence	Poor germination as seed not dried and cured properly	Various fungi attacking "cold susceptible" seeds (Table III)	—	Mice	Pigeons(?) Rooks
Seedlings and young plants	Immature seed produces wilting	Wilt (rarely)	Frit fly Wireworm	—	Rooks
Adult plants with ears	Non-adaptation to day-length may produce "tasselseeds"	Smut on ears or tasselseeds	Aphids Earwigs	—	—
Seed ripening and harvesting	Inbred seed harvesting quicker than hybrids	Various fungi on seeds harvested too late	—	Squirrels	Sparrows Pheasants
Seed storage	—	—	Moths Weevils	Mice	—

fly, although present. Earworm and borer are absent in England but frit fly, usually parasitizing oats, violently attacks sweet corn and other maize whenever possible (Cunliffe and Hodges[4]). Frit fly strains in North America and England must therefore differ genetically in potentiality to attack some crops and not others, the reason offering a challenge to evolutionists.

The life-cycle of a sweet corn crop can be divided into five stages, each involved in a particular aspect of disease and pest resistance. These are summarised in Table II. In addition, there are behaviour differences between hybrids and inbreds under English conditions (Haskell[10] [11] [12]). Abnormalities may also arise from physiological disturbances: thus wilting is associated with immature seeds as well as with susceptibility to insect attacks.

FUNGAL DISEASES

A desirable factor in England is cold tolerance, formerly known as cold hardiness or seedling blight. This is ability of seeds to germinate after lying in cold, wet soil, once thought important as it was considered advisable to sow earlier for an advanced, more profitable crop. Now it is known that sowing too early does not greatly increase earliness (Haskell[8]); sweet corn varieties are already genetically pre-determined for earliness or lateness, sowing times having comparatively little effect. Selections were therefore made to improve cold tolerance of sweet and dent corns by sowing first in March and breeding from survivors, while in later generations taking seeds from those surviving February sowing (Table III). There were successes and failures: some strains like

TABLE III

Effect of Selection on ability of Sweet Corn seed to Germinate under Cold and Wet Conditions

(after Mather & Haskell[17])

Generation of Selection	Mean % Germination			
	Canada Gold		Early Golden Market	
	Sowings in		Sowings in	
	February	March	February	March
Unselected	—	32	—	1
1	16	79	13	85
2	29	67	19	63
3	30	—	18	—

Canada Gold and Golden Standard Maize rapidly improved in tolerance, while others such as Early Golden Market and Peruvian derivatives showed little or no response (Mather and Haskell[17]). Here is an example of how straight-forward selection may or may not produce improvement, success or failure depending ultimately on the genetical constitutions of the introduced varieties. It also shows that when commencing selection for disease resistance, it is advisable to start with a wide range of material.

A review of cold tolerance is given by Wernham[20]). It is due to various fungi acting under adverse conditions for optimum germination. They kill off young embryos or seedlings before these emerge above soil level. Table IV lists fungi identified by American

TABLE IV

More Common Fungi Detrimental to Maize Germination under Adverse Conditions (Summarised from Wernham[20])

Type of fungus	Genus	Species	Activity
Oomycete	*Pythium* (All common)	*P. arrhenomanes*	—
		P. graminicola	Highly destructive
		P. debaryanum	Highly destructive
		P. ultimum	—
		P. irregulare	—
Ascomycete	*Gibberella*	*G. zeae*	Highly destructive
Fungi Imperfecti	*Diplodia*	*D. zeae*	Moderately destructive
	Penicillium	*P. oxalicum*	Moderately destructive
	Rhizoctonia	*R. solani*	Moderately destructive
	Helminthosporium	*H. maydis*	—
		H. bicolor	—
		H. carbonum	—
		H. sativum	Moderately destructive
		H. turcicum	—

(one or more can be acting simultaneously)

workers, *Pythium* species and *Gibberella* being most frequent and most destructive. Specific fungi have not yet been identified in England, but one or more of those listed in Table IV or closely related types, may be acting. Because of this, selection for better germination in one area may not be beneficial elsewhere, should another fungal strain exist there (Mather and Haskell[17])). Cold tolerance is probably not the result of selection for resistance to a specific organism, but is equivalent to multiple selection for several properties leading to the desired effect (Haskell[10])).

There are physiological bases for differences in cold tolerance. For instance, increased resistance goes with seed maturity (Rush and Neal[18]). Sweet corns, selected for tenderness, are generally but not necessarily more susceptible than dents and flints (Haskell and Singleton[14]). Possibly differences are due to pericarp thickness, thinner pericarps allowing enzymes to more rapidly leach in cold wet soil (Haskell[10]). Seed coat damage is another cause of susceptibility (Tatum and Zuber[19]). Among genetical factors, increased tolerance is more frequent in hybrids than inbreds, being a manifestation of hybrid vigour (Haskell[10]). It may even be due directly to action of the *Su*, *su* allele controlling starchy or sugary endosperms, and there are paternal as well as maternal influences. Thus polygenes and major genes involving factors for embryos and endosperms are concerned in promoting cold tolerance.

Seedlings emerge rapidly and grow away from fungal action if seeds are shown out-of-doors when soil is warmer and drier, at the end of May or early in June. Raising seedlings in soil blocks in the frameyard and transplanting is also recommended (Haskell[11]). There is chemical control by treating seeds with tetrachloro-parabenzoquinone (spergon), 2,3-dichloro-1,4-napthoquinone (phygon) and tetramethyl-thiuram disulphide (arasan). Many American seed firms treat sweet corn seeds, especially when selling in more northerly regions, and this practice should be adopted in England and in other European countries.

The only other fungal disease of importance is smut (*Ustilago zeae*), which is comparatively rare on sweet corn in England. In maize it occurs more frequently with increased use of organic fertilizers (Garrett[6]). It is often difficult to know how plants become infected; possibly frit fly damage to growing regions of young plants helps entry of spores. Any part is attacked, but manifestation is mostly by individual ripening seeds swelling to egg size. Later these burst, liberating a mass of black spores (Plate I). As sweet corn is grown for edible ears, smut is therefore economically important.

Sweet and other corn varieties differ in smut resistance (Kyle[16]); this is under genetical control (Jones[15]). The resistant strains show incompatibility of host tissue with fungal mycelium; susceptibility may be due also to incomplete husk coverage. An

association occurs between smut attacks and plants with the 'tassel-seed' condition. Thus when Connecticut inbred C 27 grew at Merton its tassels developed seeds. These were highly susceptible to smut, a condition rare on other plants. Later plants of C 27 did not develop tassel-seeds, owing to changes in day-length, and were free from smut (Plate II). Here is an example of physiological and genetical factors acting concurrently. It is unlikely that the spores had been seed borne. Plants with infected ears should be discarded and burnt before spores are liberated.

Closely associated with fungal attacks on ripening seeds is seed quality, which includes appearance as well as germinating power. It has been found in Hertfordshire that John Innes hybrid seed can be harvested over a longer period than C 13 inbred without going mouldy inside the ears (Haskell[13]). Spoiling of seeds is partly due to fungi staining them black and pink. This is another example of a hybrid having better adaptability to adverse conditions than its inbred parent.

INSECT PESTS

One hundred and twenty years ago William Cobbett noticed several pests of maize in England. He underrated their effects because of his enthusiasm for this new introduction. However, sweet corn is sometimes attacked by frit flies (*Oscinella frit* L.) with ruinous devastation. Female flies lay eggs on germinating seedlings and the grubs burrow through growing points. Varieties differ in susceptibility, for unknown reasons, but some can grow out of attacks better than others (Haskell[9]). Although intensity of attack varies between varieties, plants within a variety are liable to different degrees of injury. A few varieties show good resistance, but so far none is immune; inbreds appear to be less susceptible (Table V). There is no association with varietal maturity, judged by time ears are ready for market. However, some American breeders have produced more resistant strains than others, an example of unconscious selection or correlated heredity, as they are unconcerned with frit fly.

Frit fly attacks vary according to when varieties are sown, and peaks probably vary annually. Attacks within a crop are not related to wind direction, individual plant attack being at random.

Plate I. Sweet corn ear with ripening grains attacked by smut.

Plate II. Inbred sweet corn with "tassel-seeds" attacked by smut.

Photos by L. S. Clarke

Plate III. Sweet corn attacked by wireworms *(right)*, wireworms and frit flies *(centre)*, undamaged control plant *(left)*.

Plate IV. Inbred sweet corn plant with crinkly leaves susceptible to aphids.

Photos by L. S. Clarke

TABLE V

Susceptibility to Frit fly Attacks in open-pollinated, hybrid and inbred strains of Sweet Corn

Reaction	Assignment Number	80 open-pollinated and hybrid varieties	59 Inbred varieties
		%	%
Immune	1.0	0.0	0.0
Susceptible	1.5	0.0	1.7
	2.0	0.0	3.4
	2.5	0.0	23.7
	3.0	2.5	18.6
	3.5	12.5	32.2
	4.0	31.3	11.9
Highly Susceptible	4.5	32.5	6 8
	5.0	21.3	1.7

Figures are means of two randomized plots with rows containing 20 open-pollinated and hybrid or 10 inbred plants. Sown 13 May, 1948 at Merton, Surrey.

There is support for the advice of Blackman[1]) not to sow too many maize seeds together, as seedling clusters more likely attract frit. Seeds can be sown closely and singly in rows, and later thinned out. Also for control are methods of seedling dusting, and a possibility during future breeding of introducing locust-resistant maize strains (*cf.* Haskell[9])).

Another important insect pest of sweet corn is wireworm. This caused considerable fatal wilting at Bayfordbury in 1952 when seedlings were a few inches high. As many as three inhabited a seed or were found devouring the base of a young plant. They had little difficulty in killing off plants when jointly acting with frit (Plate III). Generally, more vigorous inbred lines appeared to suffer less than weaker ones, which were sometimes eliminated. This was partly attributed to stronger lines rapidly outgrowing damage during early growth. Damage is heavy even in soil where wireworm counts are low, so that possibly the grubs are attracted to maize seedlings. Wireworms can best be avoided by not growing sweet corn in soil recently under grass.

Aphids also attack sweet corn. They cluster around upper leaves or near to tassels and may reduce a plant's fruiting capacity. Although an occasional susceptible plant had previously occurred, badly infected lines only appeared following ear-to-row inbreeding. Many rows of Canada Gold inbreds only were highly susceptible (Plate IV). Plants of these had difficulty in unrolling leaves,

probably due to the gene 'crinkly' which may have made them more attractive to aphids; however, 'crinkly' and aphis susceptibility are not always associated. Walter and Brunson[20]) have shown how aphid resistance is under genetic control.

Earwigs often get into ear husks and cause a nuisance by dirtying silks, and making ears less attractive for market. They are especially common in hand-pollination inbred and hybrid seed plots, creeping for protection into brown-paper pollinating bags. However, their damage is superficial. Moths, and occasionally weevils, attack sweet corn seeds during storage. These are readily controlled with DDT dust.

DAMAGE BY OTHER ANIMALS

Mice invariably attacked sugary in preference to starchy seeds during ear-to-row selections for cold tolerance. They often dug up sweet corn seeds spaced six to twelve inches apart along rows, leaving unharmed adjacent rows of starchy maize. Treating grain with red lead paste did not deter mice, nor prevented them differentiating the two kinds of endosperm. Sometimes mice got among husked ears of sweet corn and starchy maize drying together in porous bags. They invariably devastated sweet ears, merely sampling the starchy ones. Here is an example of a mendelian seed character indirectly coupled with comparative susceptibility or resistance to a pest. Another rodent, the squirrel, will gnaw ears ripening on plants, and may do considerable damage to seed crops.

Several birds attack sweet corn. Rooks pull up young seedlings, seemingly attacking at random. They either like the taste of germinating seeds or perhaps are searching for wireworms. The birds leave larger plants alone. Rooks are deterred at Bayfordbury by strewing their dismembered corpses about the plots immediately after seed sowing. However, Bunting and Blackman[2]) at Oxford have not found this successful with maize. Rooks should not prove too troublesome because sweet corn is generally grown in gardens. If they do, use of transplanted seedlings in soil blocks is recommended.

Pheasants take their toll of seeds in country districts, especially when grain is ripening. Sparrows are a nuisance on ripening ears, ripping husks back and pecking at seeds on ear tips. This replaces

damage of sweet corn by red-wing blackbirds in America (Cardinell and Hayne[3]). Sparrows are especially fond of doing damage after husks have been pulled back for seed examination. Fortunately most sweet corns have their ears well covered.

FUTURE CONTROL OF DISEASES AND PESTS

Diseases and pests of sweet corn in England are now sufficiently known to permit control by methods that are either immediate or planned for the future. Both genetical and non-genetical, or environmental, methods can be applied. Non-genetical control, which gives immediate relief, means modifying the environment, like using seed treatments or not sowing until there is warm weather. As the difficulties are still present, genetical control is advisable for permanent remedy. Long term planning is thus required for breeding for resistance to adverse conditions. Breeding would embrace cold tolerance, also frit fly and wireworm resistance.

TABLE VI

Methods of Controlling Diseases and Pests of Sweet Corn

Condition	Mode of Control	
	Genetical	Non-Genetical
Cold Tolerance	Direct selection for resistant inbreds and hybrids	Sow in suitable weather; use soil blocks
Smut	Avoid tassel-seeded types	Avoid frit fly attacks
Frit Fly Attacks	Use more resistant hybrids; select resistant inbreds; introduce genes for locust resistance	Sow between attacks or in frit-free areas; use soil blocks
Wireworm Attacks	Use hybrids or vigorous open-pollinated varieties	Avoid soil previous under grass or where wireworm count is high
Seedling Wilting	Reduction by using hybrid vigour	Use mature seeds
Seed Storage	—	Dust with 5% DDT powder
Rooks	—	Place dismembered rook corpses on plots; use soil blocks
Ears damaged by birds and rodents	Use varieties with ear-tips well covered by bracts	Replace carefully bracts of examined ears

Here simple selection during production of new inbreds, may or may not be effective, depending on genetical constitutions of the original strains. The chances of selecting for cold tolerant inbreds are fairly good, but so far this has not been too successful. Selection for resistance to frit fly seems likely to be more complicated. Genes for suitable characters may have to be introduced from germplasm of other sources, and both major genes and polygenes will play a role. Table VI surveys methods of control along these lines.

Americans have severe plant quarantine laws, as the large size of their country gives diseases and pests greater opportunity for spreading. We are better off in England for guarding against introduction of virulent pests, such as European corn borer and corn earworm that has limited sweet corn in parts of the United States. But rapid transit of airplanes between North America and Europe means keeping constant watch for diseases and pests introduced from America which will attack this native American plant.

SUMMARY

A review is given of diseases and pests attacking North American inbred, hybrid and open-pollinated strains of sweet corn in England. Genetical and environmental influences are considered as problems of adaptation. Cold tolerance, susceptibility to smut, and fungi attacking ripening grain are under genetical control, but modified by environmental conditions. Analysis of these factors helps to control these diseases. Strains differ in resistance to frit fly attacks, and this is modified by sowing time. Frit flies and wireworms, acting independently or together, may limit sweet corn growing in some areas, while aphids also attack some inbred lines. Various methods of insect control are suggested. Mice and birds eat seeds, and bird damage may also limit maize growing. An outline suggests genetical and non-genetical modes of future control for diseases and insect pests when adaptating sweet corn to new climatic and other factors. These include selection for and use of resistant strains, or control by sowing at specific times.

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REFERENCES

1) Blackman, G. E., Maize as a grain crop for small-scale cultivation. Agriculture **53**, 85–89 (1946).
2) Bunting, E. S. and Blackman, G. E., An assessment of the factors controlling the productivity of maize in England. J. Agr. Sci. **41**, 271–281 (1951).
3) Cardinell, H. A. and Hayne, D. W., Corn injury by red-wings in Michigan. Michigan Agr. Exp. Sta. Tech. Bull. 198.
4) Cunliffe, N. and Hodges, D. J., Studies on *Oscinella frit* L. Notes on the resistance of cereals to infestation. Ann. Applied Biol. **33**, 339–360 (1946).
5) Dawson, C. D. R., Sweet corn in England. J. Roy. Hort. Soc. **70**, 11–119 (1945).
6) Garrett, S. D., Root Disease Fungi. Waltham, Mass. (1944).
7) Haskell, G., New sweet corn from America. Agriculture **56**, 410–415 (1949).
8) Haskell, G., Studies with sweet corn. II. Sowing dates and plant characters. Empire J. Exp. Agr. **18**, 1–7 (1950).
9) Haskell, G., Studies with sweet corn. IV. The frit fly problem. Bull. Entomol. Research **42**, 519–526 (1951).
10) Haskell, G., Genetics of cold tolerance in maize and sweet corn seed. Heredity **6**, 377–385 (1952).
11) Haskell, G., Sweet corn and maize. John Innes Ann. Rep. 1952, 10 (1953).
12) Haskell, G., Heterosis and adaptability. Report 13th Intern. Hort. Congr. **1**, 365–374 (1953).
13) Haskell, G., Harvesting inbred and hybrid sweet corn seed in England. J. Sci. Food & Agr. **4**, 433–439 (1953).
14) Haskell, G. and Singleton, W. R., Use of controlled low temperature in evaluating the cold hardiness of inbred and hybrid maize. Agron. J. **41**, 34–40 (1949).
15) Jones, D. F., Segregation of susceptibility to parasitism in maize. Am. J. Botany **5**, 295–300 (1918).
16) Kyle, C. H., Relation between the vigor of the corn plant and its susceptibility to smut (*Ustilago zeae*). J. Agr. Research **41**, 221–231 (1930).
17) Mather, K. and Haskell, G., Breeding cold hardy sweet corn for England. J. Agr. Sci. **39**, 56–63 (1949).
18) Rush, G. E. and Neal, N. P., The effect of maturity and other factors on stands of corn at low temperatures. Agron. J. **43**, 112–116 (1951).
19) Tatum, L. A. and Zuber, M. S., Germination of maize under adverse conditions. J. Am. Soc. Agron. **35**, 48–59 (1943).
20) Walter, E. V. and Brunson, A. M., Selection for aphid resistance within inbred lines of maize. J. Am. Soc. Agron. **38**, 974–977 (1946).
21) Wernham, C. C., Cold testing of corn. A chronological and critical review. Penn. Agr. Exp. Sta. Progress Rep. 47 (1951).

SOME EFFECTS OF HEAVY METALS ON PLANTS GROWN IN SOIL CULTURE

PART II. THE EFFECT OF NICKEL ON FERTILISER AND SOIL PHOSPHATE UPTAKES AND IRON AND NICKEL STATUS OF TOMATO

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In part I of this series it was shown that cobalt at toxicity levels produced symptoms of iron deficiency in tomato grown in soil culture. Moreover, total and fertiliser phosphate contents of roots and tops respectively decreased with increased cobalt treatments at the two samplings. The total iron in tomato was not found to be correlated with symptoms of iron deficiency.

In the experiment described in this paper the effect of nickel treatment was studied in a similarly designed experiment. The results are in many ways similar to those obtained for cobalt.

EXPERIMENTAL

The soil used in the factorial experiment was derived from New Red Sandstone and was similar to the one used in the previous experiment (Nicholas and Thomas[8]). It was taken from the NK (omit P) treatment under grass in a long term manurial experiment with fruit trees at Long Ashton. The soil, pH 6.5, was air dried, mixed sieved and put in 228 bitumen painted 6″ clay pots with 1.8 kg of soil per pot. The pots were arranged in 6 blocks with 18 treatments per block incorporating 3 levels of nickel at 0, 15 and 30 milliequivalents (meq.) per pot, two levels of P^{31} as $NaH_2P^{31}O_4$ equivalent to 500 and 1000 lb. superphosphate per acre and three levels of P^{32} also as orthophosphate at 0. 2 and 4 microcuries (μC) per pot. The effect of adding sodium vanadate at 0, 5 and 10 meq. was tested in another replicate at the higher carrier phosphate and higher nickel and tracer phosphate level. Two replicate treatments per block were

adjacent resulting in a 'split plot' design based on two samplings. Tomato seed, *var.* Market King, was sown in a standard compost and the plants were transplanted (1 plant per pot) into the pots containing the test soil a month after seeding. An N K Mg supplement similar to the one given in the previous experiment [8]) was supplied to the soil. The nickel treatments were applied in solution (50 ml per pot) a week after planting followed by the labelled sodium dihydrogen phosphate at two rates.

Sampling Procedure

The plants harvested 3 and 6 weeks after the application of the nickel solutions, were subdivided into tops and roots and weighed, as described previously [8]).

Chemical Methods

The tops and roots respectively were digested with nitric and perchloric acids (Nicholas [7])). Total phosphorus was determined by the vanadate method (Hanson [1])), iron by α,α-dipyridyl (Moss and Mellon [6])), and nickel by dimethylglyoxime (Forster [3])).

Radioassay

Details of this technique have been reported in a previous paper [2]).

The following notation is used in this paper Ni_0, Ni_{15}, Ni_{30} and V_0, V_5, V_{10}, indicating milliequivalent levels of metals applied per pot; P_1^{31}, P_2^{31}, the two rates of fertiliser phosphate used equivalent to 500 and 1000 lb. superphosphate per acre and P_0^{32}, P_2^{32}, P_4^{32}, the μC levels of tracer phosphate added per pot.

RESULTS

Visual Symptoms

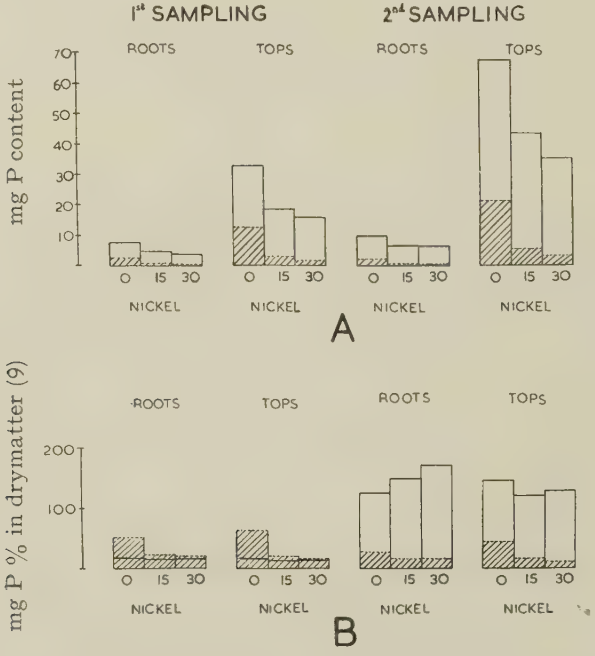
The effect of Ni on the plants after 3 weeks growth is shown in Plate I. Symptoms of iron deficiency appeared first in the Ni_{30} treatment a few days after the application of the metal. These became progressively more severe and the plants developed a severe necrosis of the leaves and petioles. The cortical tissues of the stem collapsed resulting in a bent over habit. These plants seemed to recover later and the new stem growths were upright as shown in Plate II. The phosphate level appeared to have had no effect on the severity of nickel toxicity at each metal level. This is in marked contrast to the cobalt experiment where the higher phosphate status alleviated the cobalt toxicity symptoms [8]). The addition of vanadium to this soil at 5 or 10 meq. per pot did not offset the injurious effects of nickel at the Ni_{30} rate.

Dry Weights

The statistical analysis of the dry weights of tomato (roots and tops) show that the tops only at 1st sampling increased with P_2^{31} treatment. The nickel application significantly depressed the dry weights of roots and tops at all samplings. At the 1st sampling the nickel effect was more pronounced in the tops than in the roots.

Chemical Data

The total and fertiliser P contents were determined in roots and tops respectively and in the combined parts and are expressed as total contents and as mg P per cent in dry weight. Soil P was



milliequivalents of Ni applied per pot

Fig. 1. The effect of nickel on total P and fertiliser P contents of roots and tops.

▨ Fertiliser P ☐ Soil P. (by difference)

calculated as the difference at harvest between total P content of the tops and roots combined and the sum of fertiliser P content of the tops and roots and P content of the seedlings at the time of adding the radioactive solutions to the test soil. The plants analysed

Ni_0 Ni_{15} Ni_{30}

Plate I. The effect of nickel at 0, 15 and 30 meq. per pot at the P_2^{31} level on the growth of tomato.

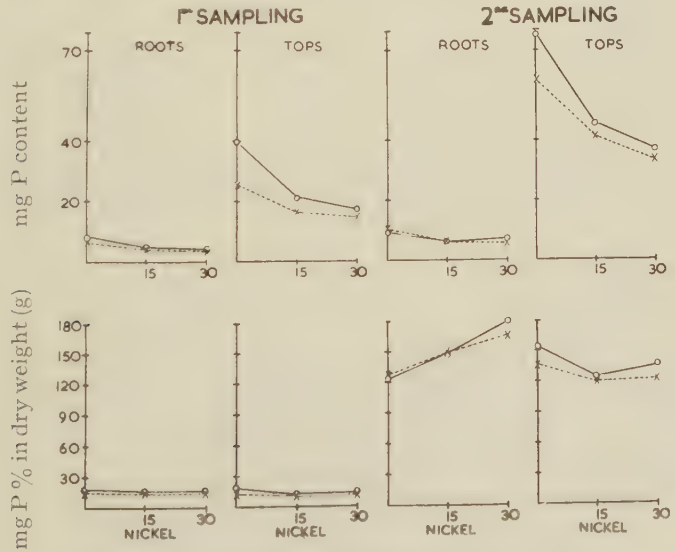
Plate II. The effect of nickel toxicity in tomato, *left*: collapse of stem resulting in bent over habit, *right*; a later recovery resulting in upright growth

for P content at this stage were of the same age as those used in the main experiment. The ratio fertiliser P/soil P contents was also determined at the two samplings to check on possible effect of the tracer on the plants (Russell, Martin and Adams[9]). The total Ni and Fe contents of tops and roots as well as their concentrations in dry matter were also determined. A summary of the main statistical results is available for inspection at Long Ashton.

1. *Total, Fertiliser and Soil Phosphate Status*

(*a*) Effect of Fertiliser Phosphate Treatment. The total P and fertiliser P contents were significantly increased at the higher level of fertiliser P treatment with the exception of total P in roots at the 2nd harvest. Soil P was affected at the 2nd sampling only (as mg P% dry matter) and in this instance there was more soil P in plants receiving the lower fertiliser P treatment.

(*b*) Effect of Nickel Treatment. The main results are illustrated in figs. 1*A* and 1*B*. The nickel treatments have signi-



milliequivalents of Ni applied per pot

Fig. 2. The effects of nickel and fertiliser phosphate treatments on the total P contents of roots and tops of tomato.

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre.

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre.

ficantly depressed the total P and fertiliser P contents of the roots and tops respectively at both samplings, presumably due to a similar reduction in dry weights of the plants. Total P expressed as mg P% in dry weight followed a similar pattern except for the roots at 2nd sampling where increased nickel treatment resulted in a higher total P concentration in the roots. The fertiliser P concentration was consistently depressed by increasing nickel treatment. Soil P was significantly reduced at both harvests by the metal, but when expressed as a concentration in dry weight soil P was affected (at $P = 0.05$) only, at the 1st harvest, and was unchanged at the 2nd harvest.

(*c*) Interaction of Nickel and Fertiliser Phosphate Treatments. Figs. 2, 3 and 4 illustrate the interactions between nickel and fertiliser P treatments on the total P, fertiliser P and soil P respectively.

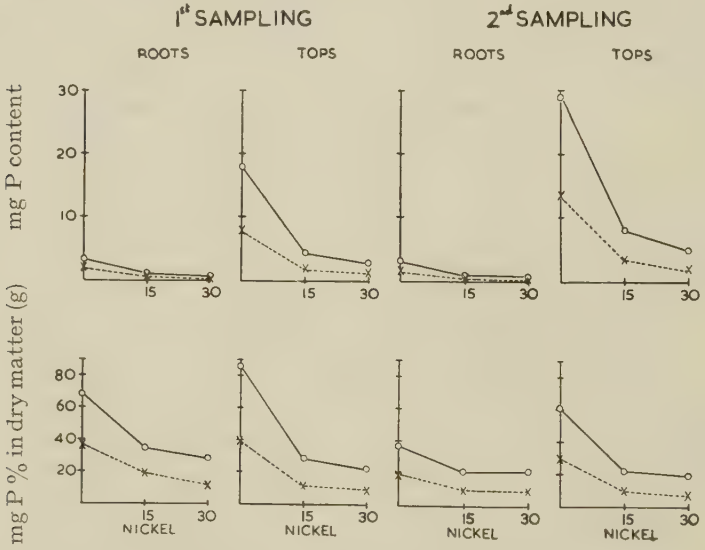


Fig. 3. The effects of nickel and fertiliser phosphate treatments on the fertiliser P contents of roots and tops of tomato.

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre.

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre.

Nickel treatment depressed P status except for total P (as mg % P) in the roots at 2nd sampling, which is significantly increased by the metal.

2. *Nickel and Iron Status*

(*a*) Effect of Fertiliser Phosphate Treatments. More total iron was present in the tops of plants receiving the higher fertiliser P treatment, but there was no difference in iron status when the element was expressed as mg per g dry weight. The nickel status was not significantly changed by P treatment.

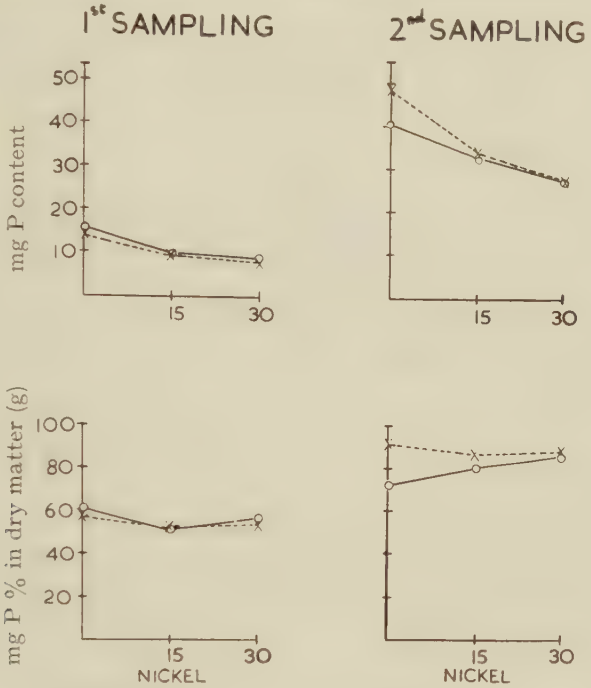


Fig. 4. The effects of nickel and fertiliser phosphate treatments on the soil P contents of tomato (whole plants).

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre.

(*b*) Effect of Nickel Treatments. Fig. 5 illustrates that the nickel treatments are reflected by the Ni status of the tops ((*μ*g Ni and *μ*g Ni g dry weight). At the 2nd sampling there is a decrease in Ni status at the 30 meq. level presumably due to root injury.

The total Fe (mg) in tops was significantly reduced by Ni at both samplings, but when the values were expressed as mg Fe per g in dry

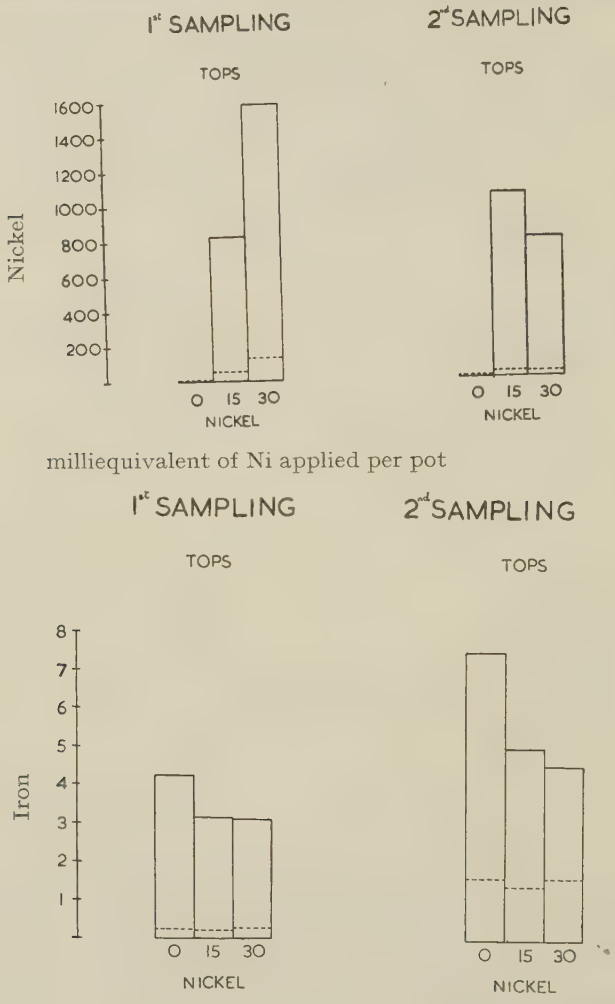


Fig. 5. The effect of nickel treatments on nickel and iron content of roots and tops of tomato.
———— total Ni (μg) or total Fe (mg)
- - - - - - - - Co (μg per g) or Fe (mg per g) dry weight

weight, the highest Ni treatment contained the most iron (1st sampling only).

(*c*) Interaction of Nickel and Phosphate Treatments. The nickel treatments were reflected at the two phosphate levels; increasing phosphate had no effect on the nickel status of the plants at a given rate of metal application.

3. *Effect of Nickel on the Redistribution of Dry Weights and Phosphate between Tops and Roots*

The effect of nickel on the redistribution of dry weights, total and fertiliser phosphate contents, between tops and roots is shown in Table I.

TABLE I

The effect of nickel treatments on the redistribution of dry weights, total phosphate and fertiliser phosphate between tops and roots of tomato plants

Functions		1st Sampling	*P*	2nd Sampling	*P*
$\frac{\text{Dry Weights Tops}}{\text{Dry Weights (Tops + Roots)}}$	× 100	$Ni_1, Ni_2 > Ni_0$	0.001	$Ni_1, Ni_2 > Ni_0$	0.001
$\frac{\text{Total P Tops}}{\text{Total P (Tops + Roots)}}$	× 100	No effect		$Ni_0 > Ni_1, Ni_2$	0.001
$\frac{\text{Fertiliser P}}{\text{Fertiliser P (Tops + Roots)}}$	× 100	$Ni_0 > Ni_1, Ni_2$	0.05	No effect	

Nickel treatment favours the redistribution of dry weight in favour of the tops. At the 2nd sampling redistribution of total P in the roots is significantly favoured by nickel treatment; as is fertiliser P (1st sampling) at 0.05 level only.

4. *Effect of Sodium Vanadate*

The vanadium salt at 5 or 10 meq. per pot did not alleviate the toxic effects of nickel as judged by dry weights, total P, fertiliser P and soil P contents, nickel and iron status of the plants.

5. *Effect of Phosphorus-32*

In accordance with a recommendation made in a previous paper (Bould, Nicholas and Thomas[2]), each carrier phosphate level was labelled with two tracer levels of low activity compatible with efficient counting, viz. 2 and 4 μC P^{32} per pot. In the data reported in this paper no significant effect was observed that could be attributed to the tracer. The critical ratio, fertiliser P uptake soil P uptake was also unaffected by either tracer level.

DISCUSSION

The present study was designed to determine whether nickel at toxicity levels in tomato would affect (*a*) the iron status as determined by visual and chemical methods, (*b*) phosphate contents and concentrations of roots and tops. The results obtained are compared with those reported in a previous experiment with cobalt [8]).

Nickel treatment resulted in an iron type chlorosis of the young leaves; a necrosis which developed quickly in stems and leaves was more severe than for cobalt excess. As a result of nickel treatment the cortical tissues of the stems collapsed and the plants were distorted. Subsequent growth however was upright (Plate II). The latter effects were not observed in tomato with cobalt toxicity. The symptoms were not alleviated at the higher phosphate treatment as for cobalt excess.

Soil P (total content) was markedly depressed by nickel treatment at both harvests but when expressed as a concentration (mg P% in dry weight) the metal effect was small (P 0.05) at the first harvest, and was not significant at the 2nd harvest. Thus, at 2nd harvest, nickel was without effect on soil P (as mg P% dry weight) although it had depressed the corresponding total P and fertiliser P concentrations. This behaviour, together with the effect of nickel in increasing the total P concentration in the roots at this harvest, may be an indication of the fixation of soil P in the roots by the metal. A similar result was also obtained for Co excess [8]).

Although the data indicate that both cobalt and nickel treatments depress fertiliser P and that, for soil P, nickel has no effect, whereas cobalt treatment increases its concentration, the fact that these effects are similar at both P^{31} levels suggests that the soil P effect is caused directly by the metal and is not due to an interaction between fertiliser P and soil P. The accumulation of soil P in the roots is of interest as it may provide a possible mechanism for the inactivation of ferric phosphate in roots as has been shown in bean plants (Biddulph and Woodbridge [1])), and in pineapple when grown in liquid culture (Sideris, Young and Krauss [10])).

Hunter and Vergnano [5]) showed that nickel toxicity in oat was enhanced when Ca, Mg, N or K supply was low or when phosphate supply was high. In experiments described herein, however, fertiliser phosphate treatment was without effect on the

incidence of nickel toxicity symptoms and reduced the effects of cobalt excess [8]).

A comparison between the Ni and Co contents (μg) and concentrations (μg per g dry weight) of tomato tops in the two experiments (Table II) shows that Co treatment is reflected at both harvests.

TABLE II

The effects of nickel and cobalt treatments on the resultant metal status of plant tops at two harvests. (The total metal contents (μg), concentrations in μg/g dry weight)

Metal level meq./pot	Metal	Ni_{15}	Ni_{30}	S.D. at $P=0.001$	Metal	Co_1	Co_4	S.D. at $P=0.001$
1st Harvest	Ni (total) μg	828	1594	252	Co (total) μg	10.7	36	14.8
2nd Harvest	Ni ,,	1060	803	221	Co ,,	19.2	74	35.4
1st Harvest	Ni (concentration) μg/g	55	131	38.7	Co (concentration) μg/g	4.2	19.5	10.5
2nd Harvest	Ni ,,	29.9	28.6	N.S.	Co ,,	4.2	17.1	8.1

At the 1st harvest there is more Ni in plants receiving the higher Ni level. The fact that Ni (as μg g dry weight) was considerably less in the nickel treated plants at the 2nd harvest compared with the 1st harvest, indicates that the absorption of the metal was probably impaired at this stage by root injury. The Ni levels in the plants are higher than those for cobalt treated plants and these reflect the greater dosages of nickel applied.

There was no positive correlation between the appearance of iron chlorosis and the iron status of the plants. In fact at the 1st sampling there was more Fe (mg per g dry weight) in plants receiving the highest nickel treatments, a phenomenon observed previously in plants suffering from heavy metal toxicity (Wallace and Hewitt [11])).

In a previous paper it was shown that the addition of molybdenum at various rates of soil did not alleviate cobalt toxicity effects in tomato [8]).

Warington [12]) showed that the incorporation of vanadium in liquid cultures containing high levels of manganese significantly increased the iron concentrations of flax shoots which was accompanied by a marked depression in growth. In the experiment described herein, the addition of vanadium at 5 or 10 meq. per pot to theNi_{30}

P_2^{31}. P_4^{32} did not alleviate nickel toxicity in tomato. Thus far no remedy has been found for the toxicity effects of cobalt or nickel produced in tomato grown in soil culture.

The precautions recommended previously in regard to the use of P^{32} in nutrition experiments were adopted [2] [8]. The tracer at 2 and 4 μC per pot did not appear to effect any of the values recroded in the appendices. The critical ratio fertiliser P uptake/soil P uptake was not changed so that it is unlikely that the tracer dosage affected the results of the nutritional experiment.

SUMMARY

1. A study has been made of the effects of nickel on the growth of tomato grown in soil culture in a factorial pot experiment. Nickel sulphate was supplied at 0, 15 and 30 meq. per pot, fertiliser phopshate equivalent to 500 and 1000 lb. superphosphate per acre. Each rate of fertiliser phosphate was labelled with the following levels of P^{32}, 0, 2 and 4 μC per pot.

2. Nickel treatment resulted first in a chlorosis of young leaves followed by a severe necrosis of stems, petioles and leaves. The stem tissues were injured and the plants were distorted. These effects were more severe than those observed for cobalt toxicity.

3. Dry weights, total P, fertiliser P contents in tops and roots were significantly reduced by nickel treatment at the two samplings, except for total P (as mg % dry weight), which was increased at 2nd sampling, roots. The latter is probably associated with a fixation of soil P in the roots.

4. The injurious effect of nickel was not alleviated by increasing fertiliser phosphate, contrary to the result obtained previously for cobalt toxicity.

5. The iron status (as mg per g) was not related to the incidence of chlorosis, in fact the iron status increased with nickel treatment.

6. The addition of sodium vanadate at 5 or 10 meq. per pot did not alleviate the injurous effects of nickel.

7. The tracer at 2 or 4 μC per pot did not effect the nutrition of the plants.

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LITERATURE CITED

1) Biddulph, O. and Woodbridge, C. G., The uptake of phosphorus by bean plants with particular reference to the effects of iron. Plant Physiol. **27**, 431 (1952).
2) Bould, C., Nicholas, D. J. D. and Thomas, W. D. E., Radiation effects with radioactive-phosphorus in barley and tomato grown in soil. Plant and Soil **5**, 36–53 (1953).
3) Forster, W. A., The determination of nickel in plant material in the presence of other metals. Analyst **78**, 560 (1953).
4) Hanson, W. C., The photometric determination of phosphorus in fertilisers using the phosphovanado molybdate complex. J. Sci. Food Agric. **1**, 172 (1950).
5) Hunter, J. G. and Vergnano, O., Nickel toxicity in plants. Ann. Applied Biol. **39**, 279 (1952).
6) Moss, M. L. and Mellon, M. G., Colorimetric determination of iron with 2 2′ Bipyridyl and with 2 2′ 2″ Terpyridyl. Ind. Eng. Chem. Anal. Ed. **14**, 862 (1942).
7) Nicholas, D. J. D., The application of rapid chemical tests to the diagnosis of mineral deficiencies in horticultural crops. J. Hort. Sci. **24**, 72 (1948).
8) Nicholas, D. J. D. and Thomas, W. D. E., Some effects of heavy metals on plants grown in soil culture. Part 1. The effect of cobalt on fertiliser and soil phosphate uptakes and the iron and cobalt status of tomato. Plant and Soil **5**, 67–80 (1953).
9) Russell, R. S., Martin, R. P. and Adams, S. N., The problem of radiation damage in fertiliser studies with labelled phosphate. Fourth Intern. Congr. Soil Sci. (Amsterdam) **2**, 138 (1950).
10) Sideris, C. P., Young, H. Y. and Krauss, B. H., Effects of iron on the growth and ash constituents of *Ananas comosus* (L) Merr. Plant Physiol. **18**, 608 (1943).
11) Wallace, T. and Hewitt, E. J., Studies in iron deficiency in crops. 1. Problems of iron deficiency and the interrelationships of mineral elements in iron nutrition. J. Pomology Hort. Sci. **22**, 153 (1946).
12) Warington, K. (Miss), Some interrelationships between manganese, molybdenum and vanadium in the nutrition of soybean, flax and oats. Ann. Applied Biol. **38**, 624 (1951).

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PRELIMINARY OBSERVATIONS ON THE DECOMPOSITION OF CHLOROPHENOLS IN SOIL

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The increasing use of synthetic organic chlorine-containing compounds in agriculture, involving their application or contact with the soil, makes a study of the action of soil-micro-organisms on such substances of some interest. Hitherto many workers have examined the bacterial decomposition of organic compounds such as aliphatic and aromatic hydrocarbons (Zobell[18]), pyridine (Moore[12]), phenol and its homologues, and naphthalene; in several cases the ability of various bacteria to utilise single aromatic compounds for their growth has been demonstrated. The only chlorine-containing aromatic compounds, which have been shown to be utilised by micro-organisms for growth, are *p*-dichlorobenzene (Erikson[6]), by species of *Micromonospora chalceae*, 2 : 4-dichlorophenoxyacetic acid (Newman and Thomas[13]), Audus [2] [3] [4]), Jensen and Peterson[10]) by some soil bacteria and α-chloronaphthalene which Tattersfield[16]) suggested may be attacked by soil bacteria. This was confirmed by Walker and Wiltshire[17]) who demonstrated that certain naphthalene-decomposing bacteria can also utilise both α-chloro- and α-bromo-naphthalene.

Since the biochemistry of the breakdown of phenol and benzoic acid by a number of Gram-negative, non-sporing bacteria has been studied extensively (Evans[7]), Happold[9]), Stanier[15]), Kilby[11]), Evans, Smith, Linstead and Elvidge[8])) it seemed logical to investigate the possible decomposition of mono-chlorophenols as a preliminary to the study of the breakdown of more complex aromatic halogen compounds.

EXPERIMENTAL METHODS

Soil percolation

Solutions of chlorophenols in tap water, with or without the addition of 10% of a stock mineral salts solution ($MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$,

0.5 g; K_2HPO_4, 2.5 g; NaCl, 1.0 g; $(NH_4)_2SO_4$, 2.5 g; $FeCl_3$, 5.0 mg in distilled water (500 ml) were allowed to percolate through columns of soil crumbs (1–3 mm). Three types of percolator were used (*a*) a simple type in which the solution, contained in a 4 litre aspirator bottle, was allowed to drop on to a layer of soil crumbs (100 g) supported on pebbles in a glass funnel, the liquid being collected in a flask and returned to the aspirator bottle once or twice a day. (*b*) A u d u s' modification [1]) of the L e e s and Q u a s t e l soil percolator and (*c*) a similar type to (*b*) but constructed from a long necked, round-bottomed flask so that the whole may be readily sterilised in the autoclave.

Estimation of Chlorophenols

Phenol or chlorophenol concentrations were estimated by the method of R e d m a n, W e i t h and B r o c k [14]). A sample (5 or 10 ml) was diluted with water (45 ml) and concentrated hydrochloric acid (5 ml) and a known excess of *N*/10 KBr-$KBrO_3$ mixture added. The mixture was allowed to stand for 2 minutes, 20% KI solution (0.5 ml) added and the liberated iodine was titrated with *N*/10 sodium thiosulphate solution using starch as internal indicator.

Soils

In most experiments, Rothamsted allotment soil, which is a light clay soil with a pH value of 6.8 was used. In other experiments soils from Exton Park, Ampthill or the Macaulay Institute were used. In all cases the 1–3 mm fraction was separated and used.

Biological Assay

The following biological method was used for detecting *p*-chlorophenoxyacetic acid in soils. A 20 g sample of soil was placed in a Petri dish, 1–2 ml water added, 20 mustard seeds were planted in it, and the sample was incubated at 25°C for three days.

Normal germination and growth of the seeds was taken to indicate the presence of only an insignificant amount of the phenoxyacetic acid derivative.

EXPERIMENTAL RESULTS

Soil Percolation Experiments

P h e n o l. The breakdown of phenol by bacteria is well known and some experiments on the decomposition of phenol under percolation conditions were carried out only for purposes of comparison. The fall in concentration of phenol when a solution is allowed to drop through soil in a simple type of percolator, using a funnel containing soil crumbs, is illustrated in the graph (Fig. 1). After a lag period of about two days, phenol disappears fairly rapidly; with an initial

concentration of 0.05%, the decomposition is complete in 2 days and with subsequent additions of 0.1% phenol solution, breakdown is complete in four or five days.

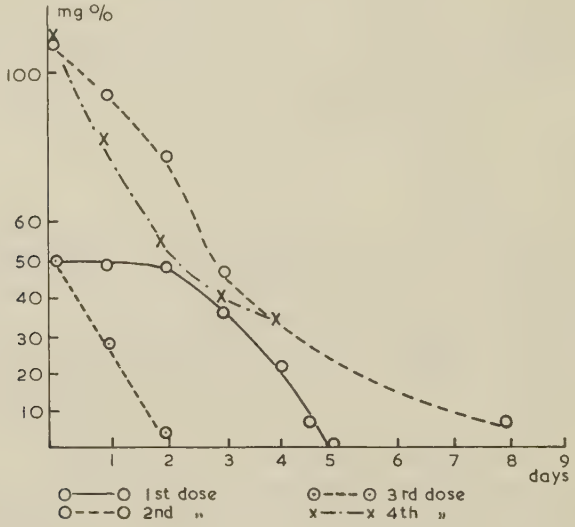

Fig. 1. Rate of loss of phenol in soil percolator.

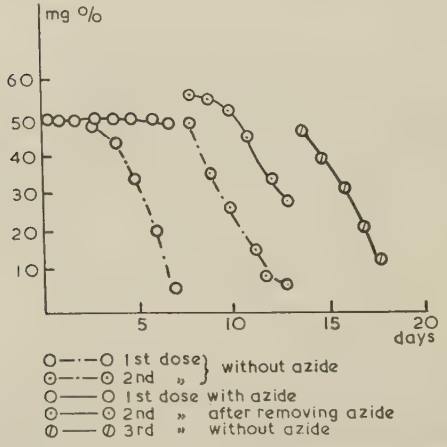

Fig. 2. Percolation of phenol with and without sodium azide through soil.

The effect of percolation of phenol solutions through soil in the modified type of A u d u s' percolator, with and without sodium azide (0.1%) is illustrated in Fig. 2. Without sodium azide, there is a

lag period of about 3 days followed by the steady disappearance of phenol and a second dose of phenol is then decomposed without any further lag period. In the presence of sodium azide, however, the disappearance of phenol is completely inhibited but when the solution is removed and replaced by a fresh phenol solution without sodium azide, there is first a lag period before decomposition of phenol commences. In these experiments, during the phase of the decomposition of the phenol, the solution became turbid as a result of bacterial growth. Bacteria capable of growth in liquid media containing only phenol as organic carbon compound, could be readily isolated by plating out.

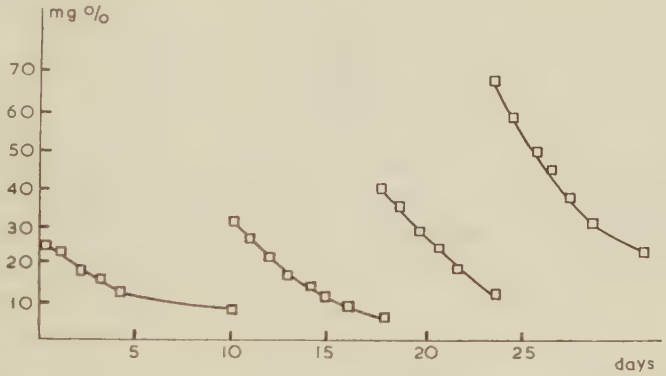

Fig. 3. Decomposition of *o*-chlorophenol in soil, showing fall in concentration after each of four doses of *o*-chlorophenol.

o-Chlorophenol. A solution of *o*-chlorophenol (1.0 g) $(NH_4)_2SO_4$ (0.5 g), KH_2PO_4 (0.3 g) and Na_2HPO_4 (0.7 g) in tap water (4 l) was allowed to percolate through Rothamsted soil in the simple type of percolator and estimations of the chlorophenol concentration were made usually every day. (The results are illustrated in Fig. 3). Further additions of *o*-chlorophenol were made at suitable intervals, and the disappearance of these subsequent doses occurred approximately twice as rapidly as that of the first dose, two thirds of which disappeared in 10 days.

Using a similar technique, the rate of disappearance of *o*-chlorophenol in fresh Rothamsted soil was compared with that in the same soil after sterilisation at a pressure of 15 lbs per sq. in. in the autoclave for 1 hour. The results showed that the fall in *o*-chlorophenol

concentration in fresh soil is about twice that in sterilised soil during 7 days percolation.

Experiments were also carried out to determine the effect of addition of ammonium sulphate on the fall in *o*-chlorophenol concentration in solutions percolating through Rothamsted soil. A slightly increased rate of breakdown of *o*-chlorophenol in the presence of ammonium sulphate was found. The loss, due to volatilisation, of *o*-chlorophenol when a 0.05% solution was allowed to drop through a glass funnel containing some glass wool, but without soil was found to be about 10% in the course of 8 days.

Effect of sodium azide on *o*-chlorophenol disappearance. Two percolators of the simple type were set up in which solutions of *o*-chlorophenol (1 part in 2500) with and without the addition of 0.1% sodium azide, were allowed to percolate through Rothamsted soil. It was found that 0.1% sodium azide was without any significant effect on the rate of disappearance of *o*-chlorophenol in soil. Similar results were obtained in a second experiment using a modified Audus' type percolator and an initial *o*-chlorophenol concentration of 1 part in 5000.

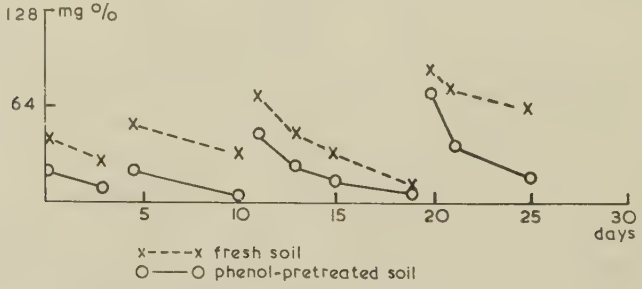

Fig. 4. Rate of loss of four successive doses of *o*-chlorophenol when percolated through fresh soil and through phenol- pretreated soil.

Percolation of *o*-Chlorophenol solution through soils pretreated with phenol. The effect of pretreating soil with phenol on the subsequent breakdown of *o*-chlorophenol was studied by using a modified soil percolator and first running two consecutive doses of phenol through the soil. Then solutions of *o*-chlorophenol were allowed to percolate and the concentration of *o*-chlorophenol was estimated at intervals. The results (Fig. 4) do not

show clearly that the development of a phenol-decomposing microflora favours attack on *o*-chlorophenol.

p-Chlorophenol. 150 ml of a solution of *p*-chlorophenol (13.3 mg 100 ml) in tap water was allowed to percolate through Macaulay soil crumbs (20 g) in an Audus' type percolator for 17 days, a further 30 mg (20 mg 100 ml) of *p*-chlorophenol was added, and after this the concentration was then estimated at varying intervals as follows:

Days	*p-chlorophenol concentration*
1	22.5 mg per 100 ml
5	19.3 mg per 100 ml
9	19.3 mg per 100 ml
13	17.6 mg per 100 ml
21	16.0 mg per 100 ml
23	13.0 mg per 100 ml

The solution in the percolator was then changed and replaced by 150 ml of a solution of *p*-chlorophenol (50 mg/100 ml) and $(NH_4)_2SO_4$ (66 mg 100 ml) and percolation was continued at 25°C. The following estimations were then made:

Days	*p-chlorophenol concentration*
1	49.7 mg per 100 ml
4	40.1 mg per 100 ml
6	38.5 mg per 100 ml
8	38.5 mg per 100 ml
11	38.5 mg per 100 ml

The solution in the percolator was then diluted by the addition of tap water (40 ml) and percolation was again continued at 25°C. The following estimations were carried out:

Days	*p-chlorophenol concentration*
1	28.9 mg per 100 ml
2	28.9 mg per 100 ml
6	28.9 mg per 100 ml
8	28.9 mg per 100 ml
10	28.9 mg per 100 ml

Thus it seems that the initial gradual fall in concentration of *p*-chlorophenol ceases and then, within the time of the experiment, the concentration remains stationary.

Percolation of *p*-Chlorophenol solution through soils pretreated with phenol. An experiment was carried out in which solutions of *p*-chlorophenol were allowed to percolate through soils previously treated with phenol solutions in a simple type of percolator. It was found that the concentration of *p*-chlorophenol fell gradually to about half its initial value in the course of 20–22 days and the rate of disappearance of a second dose was about the same as the first. The results are shown in Fig. 5 and it is clear that the disappearance of *p*-chlorophenol is much slower than that of *o*-chlorophenol and, further, there seems to be no acceleration of the rate for a subsequent dose.

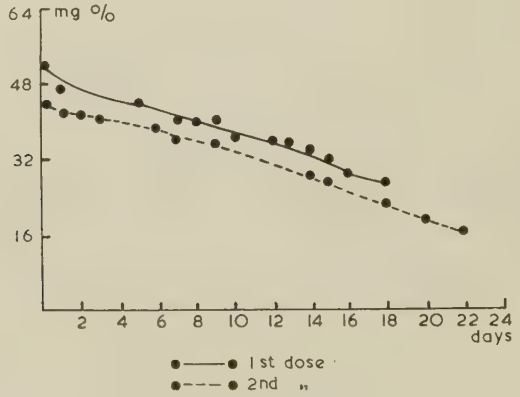

Fig. 5. Rate of loss of two successive doses of *p*-chlorophenol through phenol pretreated soils.

When the soil used in this experiment was later percolated with a solution of *o*-chlorophenol, a lag of three days was observed before the rate of *o*-chlorophenol disappearance reached that obtained in other experiments. Attempts to obtain enrichment cultures of micro-organisms, able to utilise the different chlorophenols, or to isolate such organisms on plate cultures from the soil used in the above percolation experiments were unsuccessful.

The effect of pretreatment of soils with chlorophenols on the persistence of *p*-chlorophenoxyacetic acid. Lots of air-dry Ampthill soil (750 g) were treated respectively with solutions of (I) *p*-chlorophenol, 15 mg, (II) *o*-chlorophenol, 15 mg, or (III) 2 : 4-dichlorophenol, 15 mg, in

tap water (50 ml); in each case, the solution was well mixed with the soil. A fourth sample (IV) of the same soil was kept untreated. The treated soils were stored in earthen-ware jars at room temperature (16–18) for 9 days and then similar second doses of the three compounds were added to the respective soil samples which were then stored for a further nine days. Two further doses, each of 50 mg of the three chlorophenols in tap water (50 ml) were added at 15 day intervals and finally two doses of 100 mg were added to the respective soil samples at intervals of 31 and 23 days. The soil samples were then stored at room temperature for a period of 65 days the jars being covered loosely to prevent undue drying.

The three soil samples which had received the above pre-treatments and the fourth untreated sample were given a dose of sodium *p*-chlorophenoxyacetate (50 mg) in tap water (50 ml) and then stored again at room temperature. Every few days a 20 g sample was taken from each soil and tested for the presence of *p*-chlorophenoxyacetate by planting with mustard seeds. After 24 days, the four soils received a second dose of sodium *p*-chlorophenoxyacetate (60 mg), and then 7 days later, a third dose of sodium *p*-chlorophenoxyacetate (120 mg) was added to each soil. The sampling and testing for the presence of this herbicide was also continued. The results are shown in the following table (Table I):

TABLE I

The persistence of *p*-chlorophenoxyacetic acid in soils pretreated with chlorophenols

Soil sample	Days of storage of *p*-chlorophenoxyacetate-treated soils before the seeds germinated and grew normally		
	after 1st dose	after 2nd dose	after 3rd dose
I. Pretreated with *p*-chlorophenol	No germination or growth	No germination or growth	No germination or growth
II. Pretreated with 2 : 4-dichlorophenol	No germination or growth	No germination or growth	No germination or growth
III. Pretreated with *o*-chlorophenol	22 days	7 days	7 days
IV. Untreated control	20 days	4 days	7 days

The results of this experiment show that *p*-chlorophenoxyacetate is detoxified in soil under storage conditions and the more rapid detoxification of subsequent larger doses of *p*-chlorophenoxyacetic acid suggests that this is due to biological action. Pretreatment of

the soil with *o*-chlorophenol in no way affects this phenomenon, but the failure to obtain detoxification of *p*-chlorophenoxyacetic acid in soils pretreated with either *p*-chlorophenol or 2 : 4-dichlorophenol suggests that both these compounds may persist in soil much longer than *o*-chlorophenol, and that they prevent the detoxification of the *p*-chlorophenoxyacetic acid added subsequently.

In a separate experiment, it was found that the above doses of chlorophenols, when added to soils and stored for similar periods of time, did not affect the germination of mustard seeds planted therein.

DISCUSSION

The foregoing preliminary experiments were devised to try to secure evidence as to whether mono-chlorophenols could be decomposed by biological processes. To this end, phenol itself serves as a model substance since it, in solutions of appropriate concentrations, is an antiseptic and in other solutions of not more than 0.1% concentration can serve as a carbon source of a number of bacteria. It is known (Cooper and Woodhouse[5]), that mono-chlorophenols are more potent antiseptics (*m*- > *p*- > *o*-chlorophenol, as measured by their Rideal-Walker coefficients) than phenol and so it must be anticipated that, should they be metabolised by bacteria, they would require to be in more dilute solutions. Three criteria of biological action, namely, (1) presence of a lag period during which adaptation or selection occurs, (2) loss of activity by sterilisation and (3) loss of activity in the presence of toxic agents such as sodium azide, can all be demonstrated in the case of phenol. With *o*-chlorophenol, however, only evidence of the first two criteria has been obtained, since sodium azide does not affect the disappearance of *o*-chlorophenol from the soil. With *p*-chlorophenol there is no acceleration in the rate of loss for a subsequent dose, and one might explain the disappearance of this compound as due to adsorption or to some kind of slow chemical action with soil constituents.

The disappearance of 2 : 4-dichlorophenoxyacetic acid from soil under storage conditions is known to be due to bacterial action, and it is probable that *p*-chlorophenoxyacetic acid is similarly attacked. Consequently, antiseptic treatments may be expected to prevent the decomposition of these compounds and so the failure to obtain detoxification of *p*-chlorophenoxyacetate in soils pretreated with

p-chlorophenol or 2 : 4-dichlorophenol demonstrates the persistence of enough of the latter compounds to prevent the development of bacteria capable of attacking the *p*-chlorophenoxyacetate. Pretreatment of soil with *p*-chlorophenol also causes delay in the disappearance of *o*-chlorophenol when solutions of the latter are percolated through the soil.

Since the development of a phenol-decomposing bacterial flora in soil does not promote the breakdown of chlorophenols subsequently added, it seems likely that the mode of decomposition of, say, *o*-chlorophenol, even if due to bacterial action, is at any rate markedly different from that of phenol. However, until responsible organisms can be isolated and studied more closely, little can be said of the mechanisms of the process.

SUMMARY

It has been shown that *o*-chlorophenol is much less persistent in soil than *p*-chloro- or 2 : 4-dichlorophenol and that the decomposition of *o*-chlorophenol in soil is a biological process. There is evidence that *p*-chlorophenoxyacetic acid is subject to biological decomposition in soil.

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REFERENCES

1) Audus, L. J., A new Soil Perfusion Apparatus. Nature **158**, 419 (1946).
2) Audus, L. J., The Biological Detoxification of 2 : 4-Dichlorophenoxyacetic acid in Soil. Plant and Soil **2**, 31 (1949).
3) Audus, L. J., Biological Detoxification of 2 : 4-Dichlorophenoxyacetic acid in Soils. Isolation of an Effective Organism. Nature **166**, 356 (1950).
4) Audus, L. J., The Biological Detoxification of Hormone Herbicides in Soil. Plant and Soil **3**, 170 (1951).
5) Cooper, E. A. and Woodhouse, D. L., On the Relations of the Phenols and their derivatives to Proteins. A contribution to our knowledge of the mechanism of disinfection. Part IV. The Halogen Phenols. Biochem. J. **17**, 600 (1923).
6) Erikson, Dagny, Studies on some Lake-Mud Strains of *Micromonospora*. J. Bact. **41**, 277 (1941).
7) Evans, W. C., Oxidation of Phenol and Benzoic Acid by some Soil Bacteria. Biochem. J. **41**, 373 (1947).
8) Evans, W. C., Smith, B. S. W., Linstead, R. P. and Elvidge, J. A., Chemistry of the Oxidative Metabolism of certain Aromatic Componds by Micro-organisms. Nature **168**, 772 (1951).
9) Happold, F. C., The Oxidation of Aromatic Rings by Micro-organisms in Metabolism. Biochemical Society Symposia No. 5, 85 (1950).

10) Jensen, H. L. and Peterson, H. L., Decomposition of Hormone Herbicides by Bacteria. Acta Agric. Scand. **2**, 215 (1952).
11) Kilby, B. A., The formation of β-Ketoadipic acid by Bacterial Fission of Aromatic Rings. Biochem. J. **49**, 671 (1951).
12) Moore, F. W., The Utilisation of Pyridine by Micro-organisms. J. Gen.Microbiol. **3**, 143 (1949).
13) Newman, A. S. and Thomas, J. R., Decomposition of 2 : 4-Dichlorophenoxyacetic acid in Soil and Liquid Media. Proc. Soil Sci. Soc. Am. **14**, 160 (1950).
14) Redman, L. V., Weith, A. J. and Brock, F. P., The Effect of Temperature, Acid concentration and Time on the Bromination of Phenol for Quantitative Determinations. J. Ind. Eng. Chem. **5**, 389 (1913).
15) Stanier, R. Y., Problems of Bacterial Oxidative. Metabolism. Bact. Rev. **14**, 179 (1950).
16) Tattersfield, F., The Decomposition of Naphthalene in the Soil and the Effect upon its Insecticidal Action. Ann. Applied Biol. **15**, 57 (1928).
17) Walker, N., Wiltshire, G. H., VI Congresso Internazionale di Microbiologia, Riassunti delle Communicazioni, **1**, 175 (1953).
18) Zobell, C. E., Action of Micro-organisms on Hydrocarbons. Bact. Rev. **10**, 1 (1946).